SPRING BEAUTY (*CLAYTONIA*, MONTIACEAE) IN BERINGIA: NEW EVIDENCE ON SPECIES DELINEATION FROM MORPHOMETRICS AND PHYLOGENETIC ANALYSIS OF PLASTID AND NUCLEAR DNA SEQUENCE DATA

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A

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ABSTRACT

The genus *Claytonia* L. (Spring Beauty) is well known for its attractive flowers and can be found throughout the state of Alaska. Although the genus is a highly recognizable member of Alaska flora, there is much confusion over species delimitation in *Claytonia*. This research provides additional evidence on species delineation and sectional divisions within the genus *Claytonia*. I address species delineation in *Claytonia* from Beringia using two separate and commonly adhered to species concepts. I first look at morphological species delineation using a digital approach to traditional morphometrics. I use the program ImageJ and high-resolution digital herbarium images from the University of Alaska Museum digital database, ARCTOS, to take digital measurements and quantify morphological variation in six different species of *Claytonia*. I take 20 measurements on a total of 60 specimens representing the six species. I use a hierarchal cluster analysis, principle components analysis, and conditional inference tree analysis to quantify variation in specimens. My results clearly distinguish sectional divisions, but additional measurements would be required for distinguishing species level taxa. I show that digital morphological analysis helps to enhance our understanding of morphological diversity within Beringian *Claytonia*. My second approach seeks to clarify species limits using molecular variation. I use sequences from eight different plastid and nuclear markers (nuclear ribosomal internal transcribed spacer, *trnK-matK, rps16, sqd1, at103, trnL-F* intergenic spacer, *trnS-trnG* intergenic spacer, and *ycf3-trnS* intergenic spacer) to investigate molecular variation within the genus *Claytonia*. I also provide an estimated time of divergence for these taxa. I find that sectional divisions supported by phylogenetic analysis of molecular sequences correspond with morphological variation in Beringian *Claytonia*. I also find highly supported molecular evidence

for a sister relationship between *C. joanneana* and *C. sarmentosa*. However, resolution of phylogenetic relationships within sect. *Rhizomatosae* is impaired by low genetic divergence between species indicating recent, rapid divergence. A divergence time estimate using sequence data from the genetic marker *ycf3* dates the most recent common ancestor of Beringian members of sect. *Rhizomatosae* at 3.6 million years before present. My results showing that speciation of Beringian *Claytonia* has occurred within the late Pleistocene and early Holocene may explain the lack of molecular divergence and incomplete lineage sorting within this group.

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INTRODUCTION

The genus *Claytonia* L. (Spring Beauty) is a group of fleshy herbs that is easily identified by its five showy petals, two sepalous bracts, two opposite cauline leaves, and often a basal rosette of leaves. *Claytonia* is well recognized around Alaska, but despite its charismatic appearance and long history of collection, there is much confusion around species delineation of *Claytonia* in Alaska.

The debate about the taxonomic delineation of *Claytonia* species in Alaska began during exploratory voyages of the Arctic. In 1857 Seeman published a line drawing labeled "*Claytonia sarmentosa* C.A. Mey." that he drew based on a specimen from Cape Lisburne (Seemann 1857). Many years later, in 1974 Porsild published a paper wherein he argued that the observation made by Seeman from the Cape Lisburne specimen was actually *C. arctica* Adams and not *C. sarmentosa* (Porsild 1974). In this paper Porsild included a photograph of a *Claytonia* specimen deposited at the National Herbarium of Canada (CAN). However Porsild recognized that, unlike the Russian *C. arctica*, which has white flowers with a splotch of yellow, the plants found in Alaska had pink flowers. The description of *C. arctica* plants with pink flowers marks the beginning of the term "Alaskan *arctica*", which many Alaskan botanist use to describe these plants. In 1981 Russian botanist Boris Yurtsev, who was familiar with *C. arctica* from Russia, determined that the plants referred to as "Alaskan *arctica*" were significantly different from *C. arctica* plants found in Russia and formally published a new name, *C. porsildii* Jurtzev (Yurtsev 1981).

To complicate the matter further, in 1937 Edith Scamman collected a morphologically distinct specimen of *Claytonia* on Eagle Summit near Central, Alaska that was later described as

a new species, *C. scammaniana* Hultén (Hultén 1939). In his description, Hultén noted that this species has a singly-borne flower with linear leaves and "does not seem to be very closely related to any other *Claytonia* species of the region". Since then, the name *C. scammaniana* has been applied to a variety of plants that do not match Hultén's original description. Even as soon as 1968 when Hultén published his *Flora of Alaska* he had broadened his concept of *C. scammaniana* to include a number of different plants that were not consistent with the type description of *C. scammaniana* (Hultén 1968).

Since the publication of Hultén's *Flora of Alaska* (1968) significant changes have been made to the taxonomic treatment of *Claytonia*. The genus was one of many formerly recognized as a member of the family Portulacaceae (Takhtajan 1997). However, recent phylogenetic analyses based on molecular sequencing showed Portulacaceae as formerly described to be paraphyletic (Angiosperm Phylogeny Group II 2003; Angiosperm Phylogeny Group III 2009; Chase and Reveal 2009; Nyffeler and Eggli 2010). The newly circumscribed Portulacaceae sensu stricto is comprised of only the genus *Portulaca* L., all of the remaining genera are now recognized in Anacampserotaceae, Basellaceae, Cactaceae, Didiereaceae, Halophytaceae, Montiaceae, and Talinaceae (Chase and Reveal 2009; Nyffeler and Eggli 2010). The majority of genera formerly circumscribed as Portulacaceae from Western America are now included in the monophyletic Montiaceae, which is supported by characters of vegetative morphology and molecular analysis (Hershkovitz and Zimmer 2000; Hershkovitz 2006; Nyffeler and Eggli 2010). In Alaska Montiaceae is represented by two genera: *Claytonia* and its sister genus, *Montia*. Within Alaska, the presence of five distinct petals subtended by two fleshy sepalous bracts allows quick identification of the Montiaceae (Hultén 1968; Cody 2000). *Claytonia* is easily distinguished from *Montia* by the arrangement of the cauline leaves: *Claytonia* has a single pair

of opposite cauline leaves, while *Montia* has alternate leaves that sometimes extend the whole length of the stem (Miller 2013).

Sectional divisions (sensu O'Quinn and Hufford) of *Claytonia* based on perennation structures are fairly clear and are both morphologically and molecularly supported (von Poellnitz 1932; Swanson 1966; O'Quinn and Hufford 2005). Species delineation and recognition, on the other hand, continue to be strongly debated. Many original descriptions were very narrowly conscribed and cannot in the strict sense be applied to many specimens. However, a broader interpretation of those descriptions creates overlap and results in synonymy issues. The aforementioned discussion on the taxonomic confusion of *C. scammaniana* presents a good example of such a conflict. Species distinctions in this group have been argued based on flower number, flower color, and leaf shape to name just a few.

In recent years molecular analyses have been used to provide information on phylogenetic relationships in Montiaceae. The chloroplast gene *ndhF* provided clarification at the familial level and strong support for the division of *Claytonia* from *Montia* (Applequist and Wallace 2001). Additional information on familial relationships was added by the use of several chloroplast DNA markers, including the chloroplast *rpl14-rps8-infA-rpl36* region (comprising coding and spacer sequences), the intergenic spacer *atpI-H*, and the *ndhA* intron (Ocampo and Columbus 2010). The use of the nuclear ribosomal internal transcribed spacer region of ITS and the cpDNA *trnK/matK* marker (O'Quinn and Hufford 2005) provided more information on the sectional and even species level relationships within Montiaceae. In particular O'Quinn and Hufford (2005) showed three well-supported sections within the genus: *Claytonia*, *Limnia* (Haw.) Torr. & A. Gray and *Rhizomatosae* Gray ex Poelln.

Section *Rhizomatosae* includes the majority of the species in Alaska. O'Quinn and Hufford (2005) sampled eight species from this section (*C. arenicola* Henderson, *C. arctica*, *C. cordifolia* S. Wats., *C. joanneana* Roem. & Schult., *C. nevadensis* S. Wats., *C. porsildii*, *C. sarmentosa*, and *C. scammaniana*), including the seven recognized by Miller and Chambers (2006) as well as *C. porsildii*, which Miller recognized as a synonym of *C. scammaniana*. However, phylogenetic analyses by O'Quinn and Hufford (2005) failed to completely resolve relationships between the taxa in this section.

Identifying variation or relationships between taxa can be hard enough, but agreeing on what should be considered a species is a problem in its own right. For Beringian species, there are two different approaches: a Russian approach championed by Volkova (1966) and Yurtsev (in Elven et al. 2011) and an American approach, based largely on the work of Miller (Miller 2003; Miller and Chambers 2006). The Pan Arctic Flora takes both approaches into account and comes to a consensus for nomenclature of arctic taxa (Elven et al. 2011). However, the Pan Arctic Flora makes many species determinations based largely on morphological variation, whereas many recent studies address species delineation using molecular methods (Hershkovitz and Zimmer 2000; O'Quinn and Hufford 2005; Hershkovitz 2006; Nyffeler and Eggli 2010). O'Quinn (2005) employed both molecular and morphological approaches in her dissertation on the tribe Montieae (Montiaceae).

The aim of this research is to provide a better understanding of the morphological and genetic diversity of *Claytonia* in Alaska. I use multiple lines of evidence to inform my understanding of species limits and diversity. Morphological analyses were conducted using specimens housed at the University of Alaska Museum of the North herbarium (ALA). Molecular analyses were conducted using both fresh material and material from dried herbarium

specimens. The results of this study provide new information about species delineations in this taxonomically difficult group and improve our understanding of *Claytonia* diversification in Alaska and throughout North America.

CHAPTER 1:

A digital approach to morphological analysis using the genus *Claytonia* (Montiaceae)[1]

Abstract – This project explored the use of digital images for morphological analysis using the genus *Claytonia* (Spring Beauty). Using images of *Claytonia* from the ARCTOS online digital database and the image processing software ImageJ we quantified morphological variation of *Claytonia* collections at the University of Alaska Museum of the North herbarium. Twenty characters, including quantitative morphological data and label data (such as latitude, longitude, and elevation) were coded for these specimens. Data were used to run hierarchal cluster, principle component, and conditional inference tree analyses using R. Our findings show that relationships based on morphological variation correspond with previous results based on molecular analysis, including distinct sectional divisions within *Claytonia*. We conclude that digital images can, at least in some cases, be a surrogate for traditional morphological measurements. In our study digital morphological analysis helps to enhance our understanding of morphological diversity within *Claytonia*.

[1]Jeffers, S. and S. Ickert-Bond. In prep. A digital approach to morphological analysis using the genus *Claytonia* (Montiaceae). *Systematic Botany.*

In recent years there has been a huge push for digitization of natural history collections. The National Science Foundation (NSF) has funded numerous efforts to aid in this digitization including the Advancing Digitization of Biological Collections (ADBC) program and the establishment of the Integrated Digitized Biocollections (iDigBio; Nelson 2014), a national resource aimed at the integration of multiple digitized biodiversity collections (www.idigbio.org). Herbaria are leading the charge in digitization efforts with the US Virtual Herbarium Project reporting over three million specimens databased and over one million specimens imaged to date (Barkworth and Murrell 2012). Now that progress has been made in these efforts, it is important that these digital resources be used in both research and undergraduate education (Cook et al. 2014). With over a million herbarium specimens currently associated with high-resolution digital images, this project explores the use of these digital images for morphological analysis.

There are many tools available for collecting morphological data from specimens using digital images. The study and comparison of form using shape data is known as morphometrics. Traditional morphometrics uses size, shape, and qualitative variables for a quantitative analysis of form. Geometric morphometrics analyzes the spatial arrangement biologically identifiable landmarks. Whether using traditional morphometrics via angular and linear measurements or geometric morphometrics using landmarks, shape data can provide valuable information on morphological variation within taxa (Henderson 2006). Programs such as WinFOLIA (http://www.regentinstruments.com/products/folia/FOLIA. html), LAMINA (Bylesjö et al. 2008), and LeafProcessor (Backhaus et al. 2010) offer semi-automated morphological data collection, but require isolated leaves that can be transformed into binary images. The program MorphoJ (Klingenberg 2011) allows images to be used directly from herbarium specimens but

requires landmarks that can be easily and consistently defined, which presents a problem for caespitose or fleshy plants that are often folded or warped during preparation. HerbariumLeafFinder (Corney et al. 2012a) is a program capable of automatically identifying individual leaves from herbarium specimens with only minor user interaction required in the early stages of analysis. However, this software was designed for broadleaved specimens and has difficulty distinguishing linear leaves from stems or roots.

Studies using other natural history collections have found the use of scanned images for digital measurements highly repeatable and less destructive to the specimens (Johnson et al. 2013). This electronic method not only cuts down on the physical handling of specimens but it allows for automation of data transfer and specimen recall, increasing the number of measurements and decreasing the time required for data collection (Loy and Slice 2010). In this study we use the genus *Claytonia* L. (Montiaceae Raf.) as a model to test the suitability of using images from digitized herbarium specimens to complete a traditional morphological survey and discuss the unique challenges *Claytonia* poses. We took manual measurements from digital images using digital measurement tools. These types of measurements can be obtained from any plant type regardless of habit, shape, or deformity resulting from specimen preparation.

Specimens and Species – The University of Alaska Museum of the North herbarium (ALA) currently houses over 260,000 collections of vascular and non-vascular plants, including the largest collection of Alaskan plants in the world. In 2007 we began the process of digitizing specimens allowing for high resolution images as well as label information to be accessed on our museum database ARCTOS (http://arctos.database.museum/). For this study we queried records for *Claytonia,* a fleshy herb with perennation structures that vary from bulbous to rhizomatous to fibrous; the genus covers a wide range of habitats throughout North America and eastern Asia.

Basal leaves often form in a rosette with flowering stems erect to sprawling. The fleshy leaves as well as the often thick perennation structures pose a challenge to the preservation in herbarium specimens. While some species, such as *Claytonia sarmentosa* C.A. Mey., appear morphologically congruent regardless of collection site, many species, such as *C. scammaniana* Hultén, show substantial morphological plasticity. *Claytonia* has undergone considerable taxonomic shuffling since the last comprehensive flora of Alaska (Hultén 1968), and some species that were documented as *Claytonia* in 1968 have been moved to the genus *Montia* L. Those that remain in *Claytonia* have somewhat nebulous keying characteristics. Even molecular analysis of this group offers little support or resolution for species level delineation between taxa, particularly those from Beringia (O'Quinn and Hufford 2005, Jeffers 2015). The goal of this project is to quantify and compare morphological variation in *Claytonia* using digital images from the online database ARCTOS at ALA.

MATERIALS AND METHODS

We downloaded a total of 741 high resolution images of *Claytonia* herbarium specimens from ARCTOS and added randomly located numbered points to the images using R (R Core Team 2014) to aide in randomization of measurements (Fig. 1.1). Synonymous species names were consolidated to reflect a single taxon and taxa that have been moved to other genera were excluded from this analysis. After consolidating synonyms, images were separated into species and 10 specimens for each Beringian species were selected at random for inclusion in analyses based on minimum sample size recommendations for plant functional traits (Cornelissen et al. 2003). Species with fewer than 10 specimens available were excluded from analysis.

Taxonomy – For this study we use the Pan Arctic Flora (PAF) as the authority on arctic species (Elven et al. 2011). We included six species of *Claytonia* from within the collections at ALA (Fig. 1.2), with ten specimens each representing a wide range of morphological variation as well as the three major sectional divisions in the genus: *C. tuberosa* Pall. ex Willd. and *C. eschscholtzii* of sect. *Claytonia; C. sibirica* L. of sect. *Limnia* (Haw.) Ledeb.; and *C. arctica* Adams, *C. sarmentosa*, and *C. scammaniana* of sect. *Rhizomatosae* Gray ex Poelln. *Claytonia acutifolia* Pall. ex Willd. subs. *gramnifolia* is recognized as a synonym of *C. eschscholtzii* Cham. We consolidated specimens labeled in ARCTOS as *C. acutifolia* subsp. *graminifolia* with *C. eschscholtzii* for analysis.

Measurements – A total of 20 characters were coded for the 60 specimens (Table 1.1; Appendix 1.1). We used the National Institutes of Health (NIH) free source program, ImageJ (Rasband 1997), for all measurements. Linear measurements were taken using the segmented lines tool, which allowed for measurements to be taken along curved or folded surfaces. For specimens with multiple plants mounted on a single sheet, we used only the plant closest to the random point one that had all structures necessary for measurements present (Fig. 1.1). An exception was made for basal leaves in *C. tuberosa*, which frequently loses its basal leaves during development or during specimen preparation. We did not exclude *C. tuberosa* plants from analysis if basal leaves were lacking; missing basal leaves in *C. tuberosa* were coded as zero. Basal leaf measurements were taken from the selected plant from the leaves nearest points two and three (Fig. 1.1). Cauline leaf measurements were taken from the stem on the selected plant nearest to point four (Fig. 1.1). The measurements from cauline leaf to flower and transition zone were taken along that stem from the base of the leaf. The transition zone refers to the point at which the plant meets the ground. Many members of *Claytonia* have stems that continue

underground for several centimeters before transitioning into the true root (O'Quinn 2005). Using the transition zone from above ground to underground instead of the starting point of the true root allowed for more consistent measurements. Floral number was assessed from the same stem as cauline leaf measurements. Latitude, longitude, and elevation were retrieved directly from the label data or georeferenced using GoogleEarth.

Analyses – All analyses were conducted using R (R Core Team 2014). Elevation was included as a variable in initial comparisons, but was excluded from further analysis due to the large number of specimens for which elevation data were not available. To determine the predictive power of existing species classifications (Henderson 2006), we analyzed our data using three of the most commonly used techniques: a hierarchal cluster analysis, principle components analysis, and a confirmatory analysis. Our data were scaled and translated into a distance matrix using a Euclidean method to account for the variability in range of values for our data. We then analyzed via an agglomerative hierarchal cluster analysis using the functions *scale*, *dist*, and *hclust* (R Core Team 2014) respectively to determine relationships between specimens with no a priori taxonomic assignment. Dendrograms were exported as tree files using the R package *ape* (Paradis et al. 2004). Tree files were visualized using FigTree v1.4.2 (Rambaut 2012). We performed a principle components analysis (PCA) to identify correlations between measured variables using the function *PCA* from the R package *FactoMineR* (Husson et al. 2014). We visualized our PCA using the *FactoMineR* functions *plot.PCA* and *plotellipses*. For the confirmatory analysis we used the R package *party* (Hothorn et al. 2006) and ran a conditional inference tree analysis using the function *ctree* in order to test the explanatory power of a priori species designations. Conditional inference trees were assembled for both scaled and unscaled data.

RESULTS

The hierarchical cluster analysis shows a rough separation of the three sectional divisions within *Claytonia* (Fig. 1.3). The majority of specimens from section *Limnia*, represented by *C. sibirica*, and section *Claytonia*, represented by *C. eschscholtzii* and *C. tuberosa*, appear in clades distinct from section *Rhizomatosae*. The single disjunct specimen of *C. tuberosa* that is nested in the *C. eschscholtzii* clade was the sole specimen with two basal leaves intact on the specimen. There is a single *C. scammaniana* that appears to be nested within *C. eschscholtzii* and one disjunct *C. eschscholtzii* specimen nested in sect. *Rhizomatosae*. There are also three specimens of *C. sibirica* that were sorted with sect. *Rhizomatosae*. The taxa of section *Rhizomatosae* are clearly grouped, but the analysis does a poor job distinguishing between species within that section.

Our principle components analysis (Fig. 1.4A–B) corresponds to the results of our hierarchal cluster analysis (Fig. 1.3). The ellipses in the individual factor map (Fig. 1.4A) represent the 95% confidence intervals around the centroid for each taxon. When we look at the individual factor map (Fig. 1.4a) for the first (PC1) and second (PC2) principle components, which account for a total of 66.30% of the variation within our specimens, *C. sibirica* specimens form a clearly distinguishable group. *Claytonia eschscholtzii* and *C. tuberosa* are also distinct, but the species from section *Rhizomatosae* (*C. arctica, C. sarmentosa,* and *C. scammaniana)* are difficult to distinguish. The variables factor map for PC1 and PC2 (Fig. 1.4B) shows basal and cauline leaf width (BW.1, BW.2, CW) are highly positively correlated ($r > .9$) with PC1, which accounts for 43.02% of the variation, but lack any considerable correlation ($r < |.1|$) with PC2 (Table 1.2). Flower number (FL) is also positively correlated with PC1 ($r = .76$) and shows little correlation with PC2 ($r = .07$). All other raw physical measurements (not including composite

length/width measurements) are positively correlated with PC1 to a lesser extent ($r < .9$), but also show at least moderate correlation with PC2 ($r > |.1|$) which accounts for another 23.28% of the variation within our analysis. Conversely, latitude and longitude (DEC_LAT, DEC_LONG) are somewhat negatively correlated with PC1 and PC2 (Table 1.2).

To determine the predictive power of our current classification, our conditional inference tree analysis forced all specimens into their a priori species classification. We ran the conditional inference tree analysis with both scaled (data not shown) and unscaled data with negligible differences in the output. Figure 1.5 shows the conditional inference tree with unscaled data. In this analysis our model correctly placed 10 of the10 *C. sibirica* specimens. The single *C. tuberosa* that had both basal leaves present was incorrectly placed, but our model correctly predicted the other nine. *Claytonia eschscholtzii* was also correctly determined for 9 of the 10 specimens included. Our model was unable to clearly predict species from section *Rhizomatosae*, mirroring the results from the PCA.

DISCUSSION

The work presented here is a first attempt at ALA to use solely digital images for a morphometric study. We found digital images were sufficient for distinguishing sectional divisions in the genus *Claytonia*. The individual species we sampled within sections *Limnia* and *Claytonia* (namely *C. sibirica, C. eschscholtzii,* and *C. tuberosa*) were also clearly distinguished from one another. However, distinction between species within sect. *Rhizomatosae* was lacking. Our hierarchal cluster analysis (Fig. 1.3) roughly separated sections with minor discrepancies. The three *C. sibirica* taxa that appear nested in sect. *Rhizomatosae* were likely placed there due to the measurement of juvenile leaves that were not fully expanded and thus significantly smaller

than leaves of other specimens of *C. sibirica*. The aberrant *C. scammaniana* that appears to be nested within *C. eschscholtzii* had an unusually linear cauline leaf that may account for its unusual position. Similarly, the one disjunct *C. eschscholtzii* specimen had a singularly rounded cauline leaf that may explain its odd grouping within sect. *Rhizomatosae*. And finally the disjunct *C. tuberosa* was almost certainly placed in the *C. eschscholtzii* clade due to the presence of both basal leaves, as basal leaves had fallen off of all other *C. tuberosa* specimens used in this analysis.

Results from our conditional inference tree analysis (Fig. 1.5) parallel the results from our hierarchal cluster analysis. *Claytonia sibirica* was accurately predicted for all specimens. A single specimen was inaccurately categorized for both *C. eschscholtzii* and *C. tuberosa*, likely due to the morphological oddities listed above. Once again, species within sect. *Rhizomatosae* failed to clearly separate into distinct taxonomic groups. Finally, our PCA gives us a better understanding of the relationship between our taxa and the traits measured. The first two principle components account for over 66% of the variation in our species (Table 1.2, Fig. 1.4), but it appears PC1 is largely correlated with variables that define overall size. There is also a negative correlation with latitude for PC1. Because *C. sibirica* is typically considerably larger than other *Claytonia* taxa in Alaska and because it is the most latitudinally restricted (Fig. 1.2), we ran all of our analyses again excluding *C. sibirica* to determine if it was skewing our results. Although individual PC scores change, there was little impact on the taxonomic groupings and categorization for all three analyses.

Our morphological results provide support for the sectional divisions proposed by previous molecular analysis (O'Quinn and Hufford 2005). Whether using the hierarchal cluster analysis completed with no a priori taxonomic restraint or using a PCA and conditional inference

tree which forced all specimens into predetermined taxonomic groups, our analyses successfully separated the sections *Claytonia, Limnia,* and *Rhizomatosae*. This approach to morphological analysis captured variation between sections even without the use of perennation structure classification.

The inability to use major keying characters illustrates the limitations of linear measurements in any sort of morphological analysis using herbarium specimens. Species in *Claytonia* are often identified by perennation structures (Miller 2003; O'Quinn and Hufford 2005; Miller and Chambers 2006), but perennation structures are hard to quantify with linear measurements for a number of reasons. Often the entire root is not collected intact, and many underground features are actually underground stems and not root (O'Quinn 2005). In rhizomatous species, many individuals may be clumped together on a specimen making distinguishing individual roots difficult. Also, many common botanical terms used for identification, such as spatulate or lanceolate, can be difficult to quantify with linear methods and rely heavily on user recognition rather than analytical measurement (Dickinson et al. 1987). While our results capture variation in leaf length and width, determining the overall shape of the leaf blade would require more measurements along the length of the leaf. The limitations of linear measurements can be circumvented by the addition of binary or categorical data, however this type of data collection restricts the sorts of analyses for which the data can be used. The addition of presence/absence characters has long been used in traditional morphometrics and is still frequently used to better understand relationships within taxa (Pereira et al. 2007; Rodrigues et al. 2013), but binary coding gives little additional information about the specimens. Categorical data may also be used to provide more specific information regarding shape without

the need for multiple linear measurements (Sosa 2007), but such classification can be quite subjective and can vary from study to study.

Identifying overall shapes, such as spatulate or lanceolate leaves, can be more easily accomplished using other available tools for morphological analysis. Landmark analysis uses a collection of coordinates of anatomically identifiable points to understand shape. The relationships of these points to one another provide information on linear and angular measurements. Research on developmental plasticity in *Potentilla* L. illustrated that landmark analysis could be very useful in understanding leaf shape (Klingenberg et al. 2012). This same sort of landmark approach has been widely applied to fields represented by natural history collections (Loy and Slice 2010) including entomology (Klingenberg and Zaklan 2000), ichthyology (Rüber and Adams 2001), mammalogy (Klingenberg et al. 2003; Drake and Klingenberg 2010), and archaeology (Thulman 2012). Another approach to identify overall shape is to isolate the outline of a leaf and use an algorithm to translate the outline into a binary image used for analyzing shape. Using these binary images, programs can extract leaf dimensions, area, perimeter and margin information, and even tooth density (Bylesjö et al. 2008; Weight et al. 2008; Hearn 2009; Backhaus et al. 2010; Corney et al. 2012b). However, many programs designed to assess phenotypic variation in leaves require plant parts to be selected, prepared, and photographed individually. And while the software is always improving to be able to image and identify leaves in the field (Kumar et al. 2012) or take measurements directly off herbarium specimens (Corney et al. 2012a), these tools are not yet capable of automating measurements on all specimens.

Our study of *Claytonia* illustrates how morphometrics using herbarium specimens pose unique challenges including deformation of plant materials being measured, unclear boundary

definition of features (i.e., leaves, stems, and roots), and difficulty in selection of features to be measured. While it may be easier to distinguish where one part begins and another ends on deciduous broadleaf plants, the fleshy nature and often caespitose growth of herbaceous plants like *Claytonia* means that leaves on herbarium specimens are often folded, curved, overlapping, or shriveled. Isolating a single leaf from insertion point to tip would require removal from the specimen in most cases, making programs that require a binary outline ineffective without destructive sampling. Landmark analysis requires consistent markers to be present across all specimens, which would be very difficult to identify given the large morphological variation of taxa in this study and the absence of reliable markers. Even if landmarks could be consistently identified, bending and folding of the leaves would significantly impact the results using such an analysis. Taking a morphometric approach that relies on traditional linear measurements allowed us to include all specimens with leaves and flowers present, regardless of deformities. Using the ImageJ segmented line tool for manual measurements allowed us to measure leaves and stems even when they were bent, folded, or overlapping. The use of digital images for these measurements meant that there was no damage to the actual specimen, measurements could be taken from anywhere in the world, and individual leaves used for measurements could be marked for repeatability of measurements (Fig. 1.1).

The measurements taken in this study were aimed at general quantification of size and shape, but studies could be tailored to assess differences in form between species or even between populations in greater detail. Future research will include morphological analysis that attempts to better capture the variation specifically within section *Rhizomatosae*. Future studies will explore other tools for using digital images for morphological analysis, including the use of landmarks for geometric morphometrics using digitized herbarium specimens of Vitaceae

(Ickert-Bond and Jeffers in prep.) and using the software HerbariumLeafFinder (Corney et al. 2012a) that automatically identifies leaves from herbarium specimens to study hybrid zones within Betulaceae (Jeffers, Patil, and Ickert-Bond in prep.). We will also further explore the use of manual linear measurements to quantify variation specifically in *Claytonia* sect. *Rhizomatosae*.

It is important to utilize digital image databases of herbarium specimens and doing so will not only increase the quantity of data that can be collected, but it will also reduce damage to specimens and the carbon footprint caused by such research. While it may be that not all morphological studies can be accomplished using this new digital resource, our study shows that even with the most challenging taxa, using digital images for morphological analysis can help to enhance our understanding of morphological diversity.

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TABLE 1.1. Characters and associated codes used in morphometric analyses. The codes are abbreviations of the measured morphological characters and are used consistently throughout this publication.

Code	Character description
Spp. Name	Species name for the specimen
BL1	Basal leaf 1 length from base to tip
BW1	Basal leaf 1 width at the widest point
BA1	Basal leaf 1 angle at the apex
BL2	Basal leaf 2 length from base to tip
BW2	Basal leaf 2 width at the widest point
BA2	Basal leaf 2 angle at the apex
CL	Cauline leaf length from base to tip
CW	Cauline leaf width at the widest point
CA	Cauline leaf angle at the apex
CP	The distance from the cauline leaves to the nearest flower, bud, or fruit (including pedicel)
CR	The distance from the cauline leaves to the transition zone (root)
FL	The number of flowers, buds, or fruiting bodies on a single stem.
BLW1	The length of basal leaf 1 divided by the width of basal leaf 1
BLW2	The length of basal leaf 2 divided by the width of basal leaf 2
CLW	The length of the cauline leaf divided by the width of the cauline leaf
CPR	The distance from the transition zone to nearest flower, bud, or fruit
DEC_LAT	The latitude at which the specimen was collected (in decimal form)
DEC_LONG	The longitude at which the specimen was collected (in decimal form)

TABLE 1.2. Character correlation matrix for morphological analysis. This table shows the principle component (PC) scores for the first five PCs for each variable used in the morphological analysis and the percent of variability for which each PC accounts.

	PC.1 (43.02%)	PC.2 (23.28%)	PC.3 (10.33%)	PC.4 (6.88%)	PC.5 (3.80%)
BL.1	0.70	0.50	0.36	0.01	0.18
BW.1	0.92	-0.06	0.17	-0.10	0.22
BA.1	0.54	-0.67	0.33	0.00	-0.18
BL.2	0.75	0.34	0.43	0.08	-0.15
BW.2	0.93	-0.07	0.19	0.00	0.16
BA.2	0.48	-0.74	0.34	0.15	-0.07
CL	0.46	0.73	-0.28	0.17	0.23
CW	0.93	-0.02	-0.08	-0.07	0.19
CA	0.71	-0.56	0.05	0.05	-0.20
CP	0.45	0.36	-0.23	0.52	-0.49
CR	0.87	0.24	-0.25	0.03	0.05
FL	0.76	0.07	-0.40	-0.02	-0.07
BLW.1	-0.22	0.68	0.56	0.06	0.05
BLW.2	-0.15	0.56	0.70	0.02	-0.15
CLW	-0.41	0.81	-0.08	0.22	-0.06
CPR	0.87	0.29	-0.27	0.14	-0.07
DEC_LAT	-0.60	-0.27	0.06	0.51	0.16
DEC_LONG	-0.06	-0.45	0.08	0.75	0.29

FIG. 1.1. Herbarium specimen with random numbers used for analysis. This is an example of a digital image used for morphological analysis with random numbers placed directly on the specimen image.

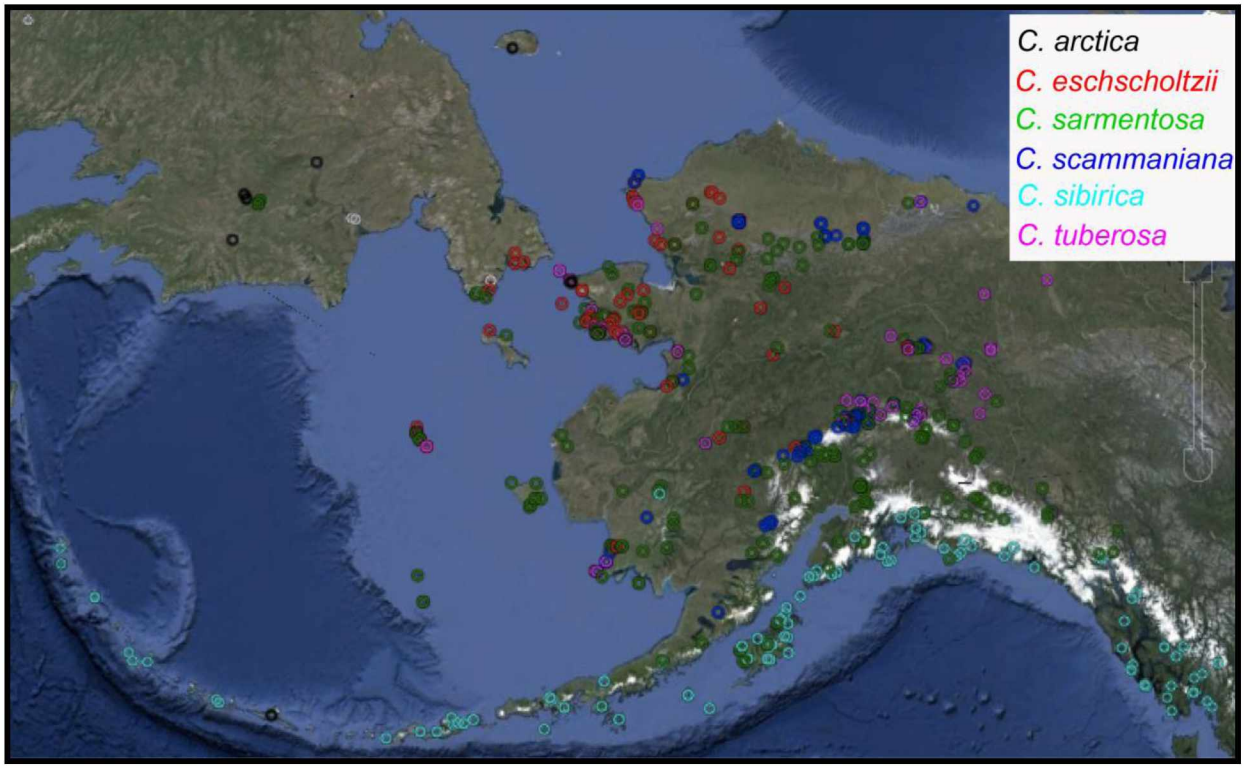

FIG. 1.2. Map of ALA specimen records of *Claytonia*. This map shows specimen records of *Claytonia* housed at ALA with the taxa represented by different colored circles: *C. arctica* (black), *C. eschscholtzii* (red), *C. sarmentosa* (green), *C. scammaniana* (blue), *C. sibirica* (turquoise), and *C. tuberosa* (magenta). Map created using GoogleEarth. Data SIO, NOAA, U.S. Navy, NGA, GEBCO. Image Landsat. Image IBCAO.

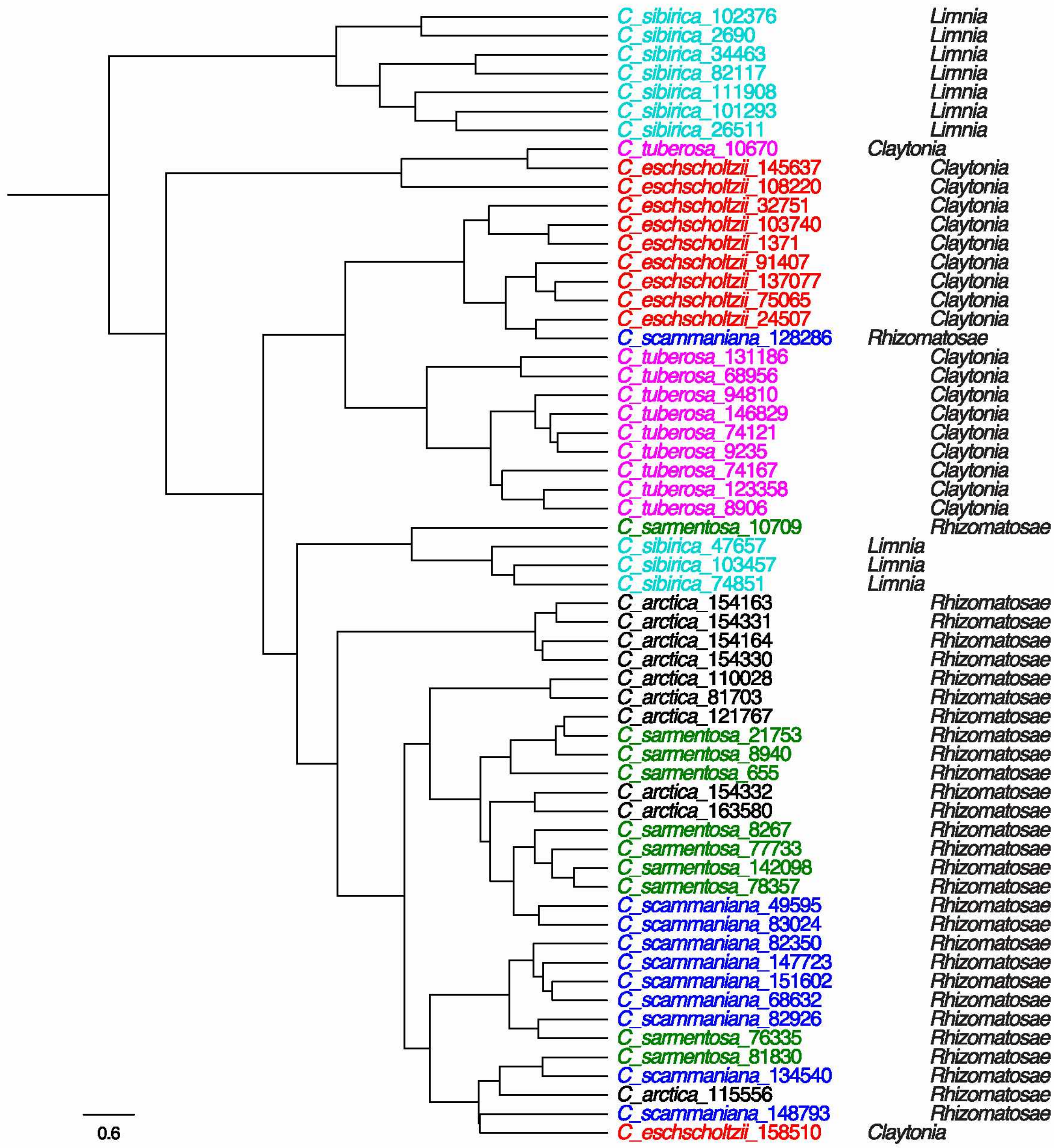

FIG. 1.3. Dendrogram showing results of hierarchal cluster analysis. Our hierarchal cluster analysis shows the major sectional divisions of *Claytonia* (noted at right) and fairly distinct clades for *C. sibirica*, *C. tuberosa*, and *C. eschscholtzii*. Specimens are labeled with their associated ALA accession number.

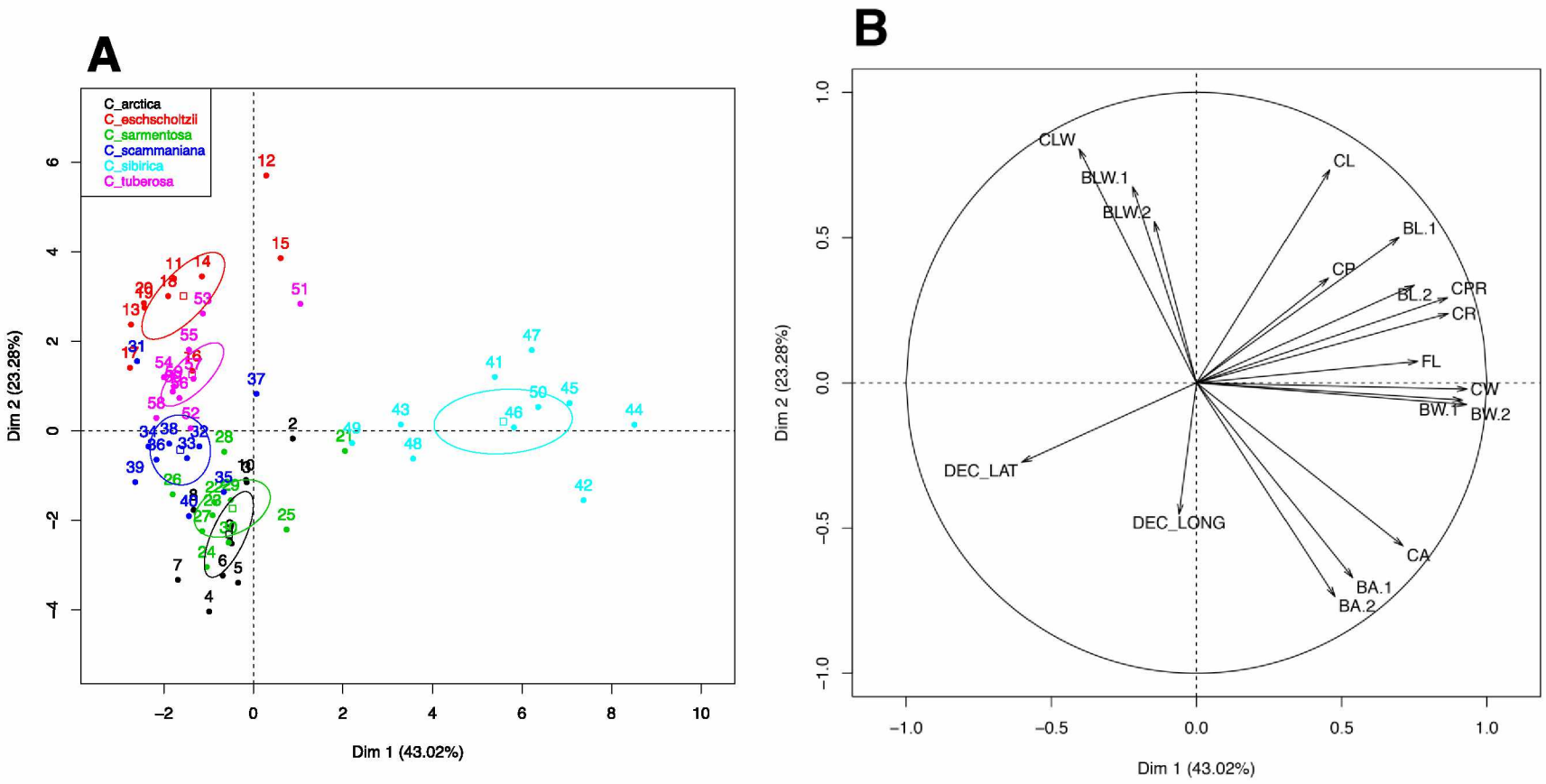

Fig. 1.4. Results of PCA for morphological variation. These graphs show results for the principle component analysis using the first and second principle components, which account for 43.02% and 23.28% of the variation, respectively. A. Individuals factor map showing ellipses for 95% CI. This map plots the individual scores for each accession used in the morphological analysis independently. B. Variables factor map. This map shows the correlation of individual measurements (i.e., basal leaf length, basal length width, flower number, etc.) with the first and second principle components.

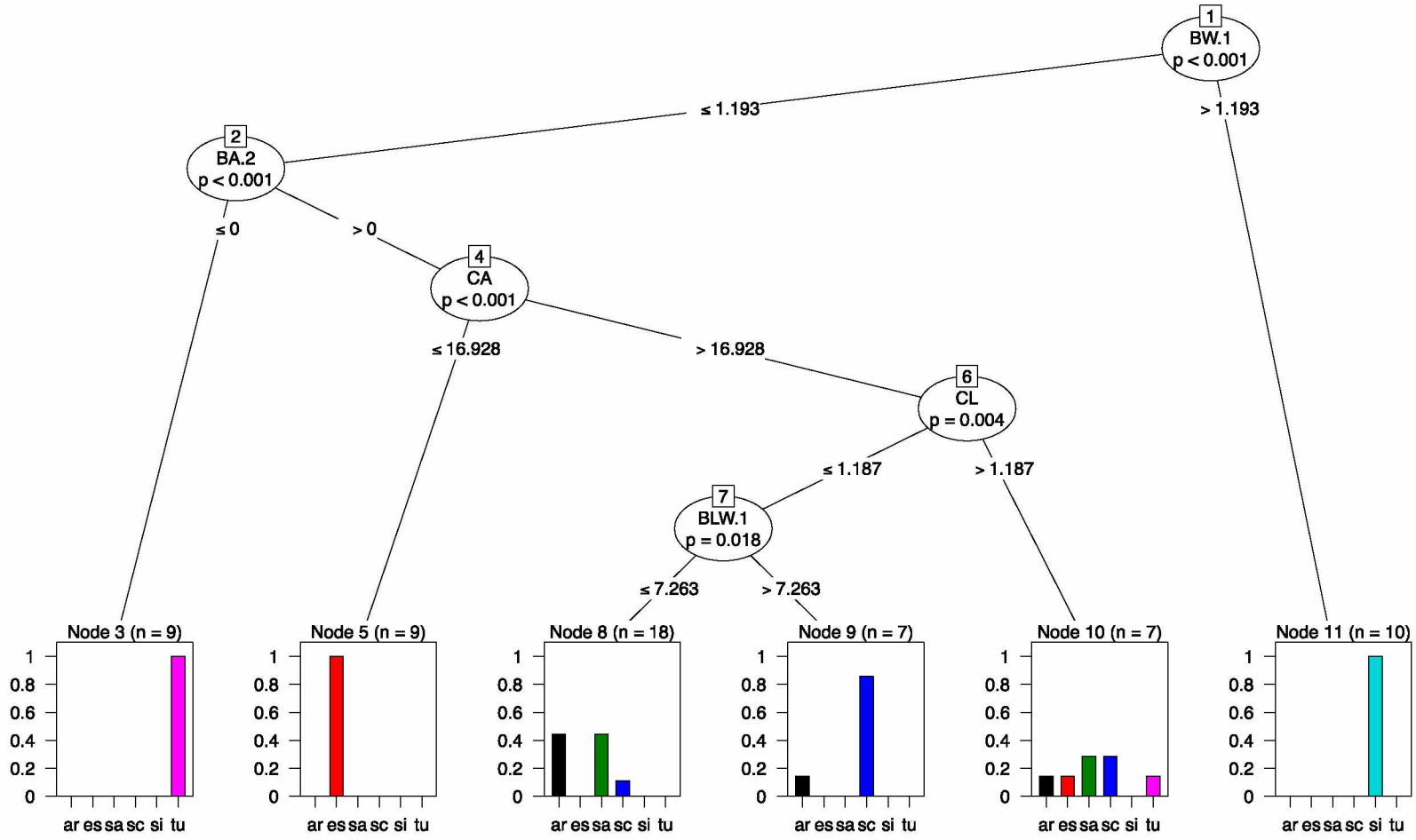

FIG. 1.5. Conditional inference tree for morphological analysis. This tree tests species as a hypothesis separating specimens based on measurements, forcing all specimens into one of the taxa: *C. arctica* (ar), *C. eschscholtzii* (es), *C. sarmentosa* (sa), *C. scammaniana* (sc), *C. sibirica* (si), and *C. tuberosa* (tu). It uses unscaled data and shows splitting criteria and p-values associated with each node, as well as the number of specimens used in the analysis for each taxon.

APPENDIX 1.1. Specimens used for measurements and character coding. Morphological measurements and label information used in the analysis are presented here. The ALA number refers to the accession number used by the herbarium at the University of Alaska Museum of the North (ALA). Angles are measured in degrees and linear measurements are in cm.

Spp.Name	ALA#	BL.1	BW.1	BA.1	BL.2	BW.2	BA.2	CL	CW	CA	CP	CR	FL	BLW.1	BLW.2	CLW	CPR	DEC LAT	DEC LONG
C. arctica	121767	2.8	0.5	101	2.9	0.6	95	0.7	0.5	62	3.5	2.2	3	5.3	4.7	1.4	5.7	65.5	-173.2
C. arctica	115556	7.1	0.7	63	7.2	0.6	53	1.7	0.8	74	4.6	6.9	6	9.9	12.5	2.2	11.5	62.5	-171.9
C. arctica	110028	3.6	0.5	83	2.9	0.5	58	1.0	0.5	55	3.7	2.4	5	7.3	6.2	2.3	6.1	52.0	-158.0
C. arctica	154163	1.3	0.4	82	1.4	0.5	97	0.8	0.6	90	2.9	2.4	3	3.0	3.0	1.3	5.3	71.7	127.3
C. arctica	154164	2.6	0.6	70	2.7	0.6	103	0.9	0.5	90	4.6	4.4	3	4.5	4.2	1.8	9.0	71.7	127.3
C. arctica	154330	3.1	0.6	89	2.7	0.7	100	1.1	0.6	52	3.7	3.2	3	5.2	3.8	1.9	6.9	71.1	127.6
C. arctica	154331	1.3	0.4	60	1.6	0.4	82	0.8	0.4	67	3.0	2.3	2	3.7	3.9	1.9	5.3	71.9	127.3
C. arctica	154332	3.3	0.3	67	2.7	0.4	68	1.0	0.4	69	3.0	3.6	3	9.4	7.3	2.6	6.6	72.2	-128.1
C. arctica	163580	3.4	0.7	95	4.4	0.5	97	0.8	0.5	67	2.6	4.7	3	5.1	9.1	1.5	7.3	70.9	-179.6
C. arctica	81703	4.3	0.7	64	3.2	0.5	52	1.2	0.7	60	2.4	3.0	3	6.0	6.3	1.6	5.4	52.2	-174.2
C. eschscholtzii	103740	8.5	0.3	10	7.9	0.3	23	2.9	0.3	9	3.1	5.0	2	27.3	29.6	8.6	8.1	63.5	-150.3
C. eschscholtzii	108220	13.7	0.5	27	11.2	0.5	18	3.9	0.3	11	9.3	8.3	2	26.8	24.2	15.3	17.5	57.5	-136.0
C. eschscholtzii	137077	5.8	0.2	12	5.5	0.2	15	1.5	0.2	9	2.1	3.2	4	30.2	26.2	7.5	5.3	67.8	-159.5
C. eschscholtzii	1371	11.3	0.4	10	11.1	0.5	10	3.2	0.4	13	2.7	4.9	3	26.0	22.8	8.2	7.7	63.4	-150.3
C. eschscholtzii	145637	14.5	0.6	18	13.0	0.7	31	3.5	0.5	15	7.0	6.8	5	24.2	18.2	7.6	13.8	64.7	-166.3
C. eschscholtzii	158510	6.8	0.5	23	6.4	0.4	11	1.9	0.4	27	4.7	3.1	2	11.0	16.1	4.7	7.8	64.9	-162.4
C. eschscholtzii	24507	6.7	0.4	17	4.8	0.3	8	1.4	0.2	16	1.5	2.4	2	15.7	18.0	7.8	4.0	68.3	-166.0
C. eschscholtzii	32751	6.2	0.4	15	9.4	0.2	14	2.5	0.3	13	3.8	3.1	2	16.5	40.3	7.3	6.9	64.8	-166.1
C. eschscholtzii	91407	6.6	0.2	10	6.8	0.3	16	2.3	0.3	17	2.7	3.5	1	32.3	19.7	8.9	6.3	65.4	-146.6
C. eschscholtzii	75065	6.8	0.2	6	6.4	0.2	8	1.7	0.2	8	2.0	5.1	2	30.9	26.4	7.3	7.2	63.7	-171.5
C. sarmentosa	10709	7.3	1.2	69	8.9	1.5	77	2.0	0.9	63	7.0	7.1	3	6.1	5.9	2.3	14.1	63.1	-145.7
C. sarmentosa	142098	2.8	0.6	66	2.4	0.4	73	0.9	0.4	47	3.0	3.0	3	4.9	6.3	2.1	6.0	59.8	-153.4
C. sarmentosa	78357	2.7	0.6	69	2.0	0.6	63	0.7	0.4	54	2.4	2.2	3	4.5	3.5	1.7	4.6	60.0	-166.0
C. sarmentosa	21753	1.8	0.5	108	1.9	0.5	97	0.6	0.4	56	2.6	1.7	3	3.7	3.6	1.5	4.3	68.1	-150.5

C. sarmentosa	655	4.1	0.8	110	3.4	0.9	97	1.1
C. sarmentosa	76335	2.4	0.3	39	2.3	0.4	47	0.5
C. sarmentosa	77733	2.6	0.4	79	2.2	0.3	72	0.5
C. sarmentosa	81830	5.4	0.3	36	6.2	0.7	71	1.2
C. sarmentosa	8267	3.8	0.6	41	3.1	0.6	72	0.7
C. sarmentosa	8940	2.9	0.7	89	2.6	0.5	95	0.9
C. scammaniana	128286	3.9	0.2	30	4.1	0.2	13	1.3
C. scammaniana	134540	3.8	0.3	36	4.9	0.3	54	1.0
C. scammaniana	147723	6.4	0.4	52	5.0	0.3	45	0.7
C. scammaniana	151602	3.8	0.2	38	3.8	0.3	39	0.8
C. scammaniana	49595	3.8	0.7	77	4.2	0.5	50	1.0
C. scammaniana	68632	3.8	0.2	23	2.9	0.3	39	0.8
C. scammaniana	148793	11.1	0.5	56	10.2	0.5	61	1.6
C. scammaniana	82350	3.5	0.3	18	4.3	0.3	20	0.8
C. scammaniana	82926	2.7	0.3	29	1.8	0.3	56	0.7
C. scammaniana	83024	3.4	0.5	83	3.1	0.6	69	0.7
C. sibirica	101293	15.1	1.8	68	14.3	1.8	41	3.2
C. sibirica	102376	8.2	2.1	133	23.9	2.1	96	1.5
C. sibirica	103457	10.2	1.6	71	9.9	1.3	53	2.8
C. sibirica	111908	10.3	1.9	83	15.5	2.4	104	3.7
C. sibirica	26511	17.7	2.2	90	18.4	1.7	76	1.8
C. sibirica	2690	9.1	1.3	70	10.8	1.0	87	2.2
C. sibirica	34463	19.0	2.2	63	11.3	2.0	51	4.6
C. sibirica	47657	9.7	1.6	60	9.2	1.8	81	2.6
C. sibirica	74851	10.5	2.0	52	9.1	1.5	64	2.6
C. sibirica	82117	18.5	3.0	81	6.9	2.1	86	4.1
C. tuberosa	10670	11.3	0.5	29	12.6	1.1	35	3.8
C. tuberosa	123358	0.0	0.0	0	0.0	0.0	0	2.4
C. tuberosa	131186	8.3	0.3	14	0.0	0.0	0	3.1
C. tuberosa	146829	0.0	0.0	0	0.0	0.0	0	2.7
C. tuberosa	68956	7.5	0.3	24	0.0	0.0	0	2.2

0.6	65	4.9	4.3	5	5.0	3.8	1.8	9.2	63.6	-149.6
0.3	39	1.7	2.5	3	7.2	5.4	1.6	4.1	62.1	-153.5
0.4	71	2.3	3.3	2	6.2	6.9	1.5	5.6	63.2	-150.3
0.6	60	3.7	6.1	3	18.9	9.6	2.2	9.7	69.5	-149.4
0.7	58	3.3	4.0	4	6.7	5.0	1.1	7.3	63.4	-150.3
0.6	57	2.5	2.0	4	4.5	4.8	1.5	4.5	63.6	-149.7
0.2	23	2.1	3.2	2	17.6	22.4	8.5	5.3	61.9	-155.2
0.4	47	4.1	4.5	4	11.3	14.2	2.7	8.6	68.3	-158.5
0.3	56	1.5	4.1	1	14.8	15.2	2.4	5.6	63.3	-149.8
0.3	27	1.6	2.9	1	15.5	14.2	2.7	4.5	65.0	-142.8
0.5	47	2.3	3.5	5	5.8	8.8	2.0	5.8	63.5	-146.3
0.4	52	1.6	1.7	1	16.9	8.9	2.0	3.3	62.7	-152.5
0.5	55	3.4	7.5	1	21.9	20.0	3.1	10.9	62.4	-152.9
0.3	57	1.4	3.3	4	13.2	14.5	2.4	4.7	63.9	-147.5
0.3	25	1.7	1.6	1	9.2	6.9	2.5	3.2	68.3	-158.3
0.3	26	2.0	1.7	4	6.8	4.9	2.1	3.8	68.3	-158.3
1.7	69	4.7	15.1	14	8.4	7.8	1.9	19.7	55.3	-131.7
2.6	161	5.4	19.0	8	4.0	11.4	0.6	24.3	56.8	-133.0
1.5	66	4.0	10.9	7	6.4	7.4	1.8	15.0	56.4	-135.0
3.6	113	5.2	24.5	17	5.6	6.4	1.1	29.6	58.6	-152.7
1.4	104	5.1	26.0	12	7.9	10.8	1.3	31.1	57.2	-153.3
2.1	132	6.1	24.4	9	7.2	10.4	1.1	30.5	53.2	-168.4
2.8	71	5.1	14.7	8	8.8	5.6	1.6	19.8	51.9	-176.8
2.0	70	1.0	16.3	5	6.2	5.2	1.3	17.3	59.5	-150.5
1.1	59	2.5	7.5	3	5.2	6.2	2.3	10.0	58.3	-134.4
2.8	57	3.5	21.0	6	6.2	3.3	1.5	24.5	59.5	-150.5
0.8	22	6.0	9.2	4	24.2	11.0	5.0	15.2	65.4	-146.5
0.8	49	4.4	5.8	6	0.0	0.0	3.0	10.1	64.6	-160.6
0.4	21	4.9	9.3	5	27.6	0.0	7.4	14.1	63.8	-145.8
0.3	19	3.0	11.0	4	0.0	0.0	8.9	14.0	64.7	-143.3
0.3	27	2.6	8.3	5	25.6	0.0	6.8	10.9	59.0	-161.7

C. tuberosa	74121	0.0	0.0	0	0.0	0.0	0	2.2	0.4	27	3.9	11.2	4	0.0	0.0	5.7	15.1	64.7	-164.0
C. tuberosa	74167	0.0	0.0	0	0.0	0.0	0	2.9	0.5	20	5.9	6.8	8	0.0	0.0	5.9	12.7	64.7	-165.8
C. tuberosa	8906	0.0	0.0	0	0.0	0.0	0	2.5	0.6	28	3.4	5.1	3	0.0	0.0	4.4	8.5	63.6	-148.3
C. tuberosa	9235	0.0	0.0	0	0.0	0.0	0	3.0	0.4	28	4.0	9.4	5	0.0	0.0	6.8	13.3	68.1	-165.5
C. tuberosa	94810	0.0	0.0	0	0.0	0.0	0	2.0	0.3	29	3.8	8.1	8	0.0	0.0	8.1	11.9	64.7	-165.3

CHAPTER 2:

Phylogenetic analyses of Beringian members of *Claytonia* section *Rhizomatosae* (Montiaceae) impaired by low genetic divergence between species indicating recent divergence[1]

Abstract – Previous phylogenetic analyses of *Claytonia* (Montiaceae) have shown strong support for existing sectional divisions within the genus. However, within section *Rhizomatosae*, the most speciose section in Beringia, interspecific relationships remain unclear and unsupported. To better understand relationships within *Claytonia* section *Rhizomatosae*, this project sampled 58 Beringian specimens of *Claytonia* and outgroup taxa in Montiaceae. We used DNA extracted from both field-collected and herbarium specimens deposited at the University of Alaska herbarium. Using sequences from both nuclear and plastid markers (nuclear ribosomal internal transcribed spacer, *trnK-matK, rps16, sqd1, at103, trnL-F* intergenic spacer, *trnS-trnG* intergenic spacer, *ycf3-trnS* intergenic spacer) we inferred phylogenetic trees with maximum likelihood, maximum parsimony, and Bayesian inference. Our results provide further support for sectional divisions in *Claytonia*, but species level relationships remain largely unresolved. We do recover a strongly supported clade with *C. joanneana* as sister to *C. sarmentosa*, which is contrary to the hypothesis posed in the Pan Arctic Flora. Our estimate of divergence times infers the split of Beringian members of section *Rhizomatosae* from the rest of *Claytonia* at around 3.6 MYA. This recent divergence contributes to the lack of clear phylogenetic resolution in the Beringian members of *Claytonia* section *Rhizomatosae*.

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[1] Jeffers, S. and S. Ickert-Bond. In prep. Phylogenetic analyses of Beringian members of *Claytonia* section *Rhizomatosae* (Montiaceae) impaired by low genetic divergence between species indicating recent divergence. *Systematic Botany*.

Swedish botanist Eric Hultén who wrote the most widely used text for floristics in Alaska, *Flora of Alaska* (Hultén 1968), is also well known for his work in arctic biogeography. Hultén was instrumental in our understanding of arctic species and speciation, and he is credited for coining the term "Beringia" (Hultén 1937; Abbott and Brochmann 2003). He used this term to refer to the area he proposed stayed ice-free during the Quaternary glaciations that spans from the Lena River in northeast Russia (125° E. latitude) and the Mackenzie River in northwest North America (130 ° W. longitude), and from the Arctic Ocean in the north to mountains in southern Siberia and Alaska in the south (Hultén 1937; Abbott and Brochmann 2003). Since then many studies have shown that this region, Beringia, served as a refugium for many of the alpine and arctic species present in Alaska today (Yurtsev 1982; Abbott and Brochmann 2003; Abbott and Comes 2004; O'Quinn and Hufford 2005; Provan and Bennett 2008; Beatty and Provan 2010; DeChaine et al. 2013). Despite the pivotal role of Beringia in the preservation of the arctic flora during the last glacial maximum, the vascular plant flora of the Arctic is remarkably species poor with only 1% of the world's known vascular plant species occurring in the Arctic (Daniëls et al. 2013).

While Beringia and the Arctic flora may be relatively species poor, they are nevertheless home to many unique and endemic species. The genus *Claytonia* L. (Montiaceae) as currently recognized is comprised of about 27 species found in North America and eastern Asia (Miller and Chambers 2006). *Claytonia*, commonly known as the Spring Beauty, is typically found in moist, high elevation habitats on rocky outcrops or scree slopes and is widespread throughout high northern latitudes, with a third of those species present in Beringia (O'Quinn and Hufford 2005). However, there has been a long-standing dispute regarding the taxonomic identity of *Claytonia* worldwide and about which species occur in Alaska. Previously, the genus *Claytonia*

was recognized in the family Portulacaceae (Takhtajan 1997). This family as previously recognized is paraphyletic (Applequist and Wallace 2001, Angiosperm Phylogeny Group II 2003); and taxonomic revisions have been made to recognize eight monophyletic families (Angiosperm Phylogeny Group III 2009; Chase and Reveal 2009; Nyffeler and Eggli 2010).

The genus *Portulaca* L. is the sole remaining representative of the newly circumscribed Portulacaceae, all other genera are now recognized in Anacampserotaceae, Basellaceae, Cactaceae, Didiereaceae, Halophytaceae, Montiaceae, or Talinaceae (Nyffeler and Eggli 2010). Several recent molecular studies have informed our understanding of interfamilial relationships in Portulaceae sensu lato. Hershkovitz (2006a) used the chloroplast DNA *ycf3-trnS* intergenic spacer (IGS) and the nuclear ribosomal DNA internal transcribed spacer (ITS) region to resolve relationships within Portulaceae sensu lato, later Nyffeler and Eggli (2010) provided a detailed revised classification of Portulaceae s.l. by using sequence data from *cp*DNA markers including the chloroplast *rpl14-rps8-infA-rpl36* region, *matK*, *ndhF*, the intergenic spacer *atpI-H*, and the *ndhA* intron.

In Alaska only Montiaceae occurs, represented by two genera: *Montia* L. and *Claytonia* L. (O'Quinn and Hufford 2005; Elven et al. 2011). Research on the tribe *Montieae* (Montiaceae) using the *nr*DNA ITS and the *cp*DNA *trnK* intron and the 5' end of the *matK* coding region (*trnK/matK*) offered robust support for the monophyly of genera and even supported the recognition of distinct sections within the genus *Claytonia*: sect. *Claytonia,* sect. *Limnia* (Haw.) Ledeb., and sect. *Rhizomatosae* Gray ex Poelln. (O'Quinn and Hufford 2005). This molecular evidence roughly corresponds with sectional divisions that have been characterized by perennation habit: bulbous (to thick taproot), fibrous, and rhizomatous respectively (Swanson 1966; O'Quinn and Hufford 2005). Previous taxonomic treatments (von Poellnitz 1932; Swanson

1966; McNeill 1975) have also recognized a fourth section, sect. *Caudicosae* A. Gray ex Poelln., whose members showed a variety of perennation habits; but taxa previously recognized in this section have been redistributed among the other three by O'Quinn and Hufford (2005) based on molecular evidence. Section *Rhizomatosae* includes the majority of the species in Beringia (as well as the western North American *C. cordifolia* S. Watson, *C. nevadensis* S. Watson, and *C. arenicola* L.F. Hend.), but analyses to date have failed to resolve phylogenetic relationships within this section.

When dealing with taxa present in Beringia, there are two independent taxonomic concepts to delineate *Claytonia* species. For the most part previous molecular analyses, including the treatments listed above, follow the North American concept proposed by Miller (Miller 2003; Miller and Chambers 2006). The Russian concept proposed by Volkova and later championed by Yurtsev (Volkova 1966; Elven et al. 2011) differs in many aspects from that of Miller, particularly in regards to the taxonomic status and recognition of *C. acutifolia, C. arctica, C. eschscholtzii, C. porsildii,* and *C. scammaniana*. The Pan Arctic Flora (Elven et al. 2011) takes both approaches into consideration and proposes a treatment that can be used across Beringia. Here we follow the Pan Arctic Flora (PAF) when referring to arctic species. For the remainder of the species we accept the names on file at the University of Alaska herbarium (ALA), which follows the naming convention of the *Flora of North America* (FNA; http://floranorthamerica.org/). This means that some specimens which are listed in ALA using FNA nomenclature (Miller 2003) may be treated as a different species in the current analysis. We also include an additional taxon that has been identified as a putative new species from the serpentine barrens near Feniak Lake in Noatak National Preserve (NOAT). Originally collected by Steve Young (1974), who designated a type specimen for "*C. noatakensis*", should a new

species be described. Both David Murray, former curator of the ALA herbarium, and Carolyn Parker, long-time research associate at ALA, who are very familiar with the Alaska flora have agreed that specimens from the Feniak Lake population look morphologically distinct, but have yet to offer a formal description (D. Murray, ALA, Fairbanks, personal communication, March 2011; Carolyn Parker, ALA, Fairbanks, personal communication, May 2011).

To gain a better understanding of the genetic diversity within *Claytonia* sect. *Rhizomatosae* and of relationships between Beringian taxa of Montiaceae, this project sampled 58 accessions of 10 recognized species as well as one taxon argued to be a distinct species in Beringia not recognized in previous analyses (i.e., *"Claytonia noatakensis"*). A total of eight molecular markers were used in an attempt to resolve species level relationships within Beringian *Claytonia*. Following marker selection used by O'Quinn and Hufford (2005) for phylogenetic analysis of the tribe *Montieae* (which includes *Claytonia*), we amplified the *nr*DNA ITS and the plastid-encoded *trnK/matK*. In addition, we also amplified the cpDNA *ycf3-trnS* IGS employed by Hershkovitz (2006b) for differentiation at the generic level.

In an attempt to overcome the potential lack of resolution in previous studies, we also employed the *rps16* gene (Oxelman et al. 1997), which has shown promising results at resolving species level relationships in plants (Shaw et al. 2005; Martirosyan et al. 2009), including many arctic plants like *Lagotis* Gaertn. (Plantaginaceae), *Silene* L. (Caryophyllaceae), and *Primula* L. (Primulaceae) (Popp et al. 2005; Guggisberg et al. 2009; Li et al. 2014). Similarly, several *cp*DNA IGS have been shown to be highly variable (Curtis and Clegg 1984; Palmer et al. 1988), including the IGS between *trnS-trnG* (Hamilton 1999) and *trnL-F* (Taberlet et al. 1991). Finally, this project used two low copy nuclear Conserved Ortholog Set (COS) markers, *at103* and *sqd1*, in order to provide resolution at the lowest taxonomic levels (Li et al. 2008). The objectives of

this study are to reconstruct phylogenetic relationships within Beringian members of *Claytonia* section *Rhizomatosae* and to provide a time estimate for species divergence in this group.

MATERIALS AND METHODS

Taxon Sampling – We sampled 58 accessions of Montiaceae, including both *Claytonia* and *Montia* (Appendix 2.1) with special emphasis on the Beringian members of sect. *Rhizomatosae*. We included 10 species in our analysis: *C. arctica* Adams, *C. eschscholtzii* Cham., *C. joanneana* Roem.& Schult., the putative "*C. noatakensis*" (Young 1974), *C. porsildii* Jurtzev, *C. sarmentosa* C.A. Mey., *C. scammaniana* Hultén, *C. tuberosa* Pall. ex. Willd., *M. chamissoi* Tidestr., and *M. fontana* L.. Original fieldwork included collection of whole organism voucher specimens throughout Alaska including Eagle Summit, Feniak Lake (NOAT), Hatcher's Pass, and duplicates of most prior collections accessible along the main road system (Appendix 2.1). Genetic material was also obtained from herbarium specimens on file at ALA and sequences from previous studies were downloaded from GenBank (Appendix 2.1).

Extraction and Amplification – We extracted total DNA from 58 specimens using DNeasy Plant Mini Kit protocols (Qiagen Inc., California). All PCR amplifications were initially done using the same "cocktail" recipe. The PCR mix was prepared directly before amplification and included 12.5 µl REDTaq DNA Polymerase (Sigma-Aldrich), 5.5 µl water, 1 µl primer I, 1 µl primer II, and 1 µl DNA. For some COS products that did not amplify well, we increased the DNA amount by 1 µl. For species with more than four accessions we restricted full genetic sequencing to the four accessions that amplified well consistently. Primer details are listed in Table 2.1 and thermocycler conditions are provided in Table 2.2. PCR products were visualized

using gel electrophoresis and ethidium bromide (EtBr) staining/imaging before preparation of amplicons for sequencing.

Sequencing and Analysis –_PCR products were sent for cleanup and Sanger sequencing to the High Throughput Genomics Center (Seattle, Washington). We evaluated sequences using Sequencher v4.7 (Codes 2006) and trimmed to >90% confidence for base pair recognition. Sequences were aligned using MUSCLE v3.8.3 (Edgar 2004) and alignments were visually inspected using Mesquite v3.01 (Maddison and Maddison 2001).

Phylogenetic analyses were performed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) for each marker individually as well as concatenated by genome. Maximum likelihood (ML) analyses were run using RAxML version 8 (Stamatakis 2014) using the RAxML-HPC2 on XSEDE and implemented on the CIPRES Science Gateway computational portal (Miller et al. 2010). Analyses were run using default simple parameters and a random seed value was entered for a starting tree. Statistical support was measured by ML bootstrapping with 1000 rapid bootstrap replicates.

MrModeltest+PAUP* (Nylander 2004) was used to determine the optimal model of sequence evolution for each MP and BI analyses based on Akaike Information Criterion (AIC) scores. Maximum parsimony (MP) searches were run in PAUP* 4.0b10 (Swofford 1998), using a bootstrap method with heuristic searches of 100 replicates to generate a bootstrap 50% majority-rule consensus tree. BI analysis was conducted using MrBayes 3.2.0 (Ronquist et al. 2012). Each Markov Chain Monte Carlo (MCMC) analysis used four runs, with four chains each, for 10 million generations with a .25 burn-in fraction with a sampling frequency of 1,000 generations. The remaining trees were pooled to calculate the majority-rule consensus tree with average branch lengths and posterior probabilities. For the combined analysis we partitioned the

data a priori and used models of sequence evolution as previously determined in Mr.Modeltest based on AIC scores. A reduced nuclear concatenated dataset (Fig. 2.9b) was constructed by removing accessions for which ITS was not successfully sequenced (removing 414_*C.eschscholtzii*, 12_*C.scammaniana*, and 448_*C.noatakensis*). Phylogenetic trees were visualized using FigTree version 1.4.2 (Rambaut 2012).

Divergence Time Estimation – We estimated divergence times using programs included in the Bayesian Evolutionary Analysis Trees package (BEAST) v1.8.1 (Drummond et al. 2012). Our analysis used an expanded sample to cover the diversity within Montiaceae including taxa from O'Quinn and Hufford (2005) and Hershkovitz (2006a, 2006b) using sequence data from GenBank (AY764037–AY764087, DQ497995–DQ498057, AF084138–AF084158, DQ090364–DQ090397). Data were combined into matrices by marker in Mesquite v3.01 (Maddison and Maddison 2001) and aligned using MUSCLE v3.8.3 (Edgar 2004). Our xml file was prepared using BEAUti v1.8.1 (Drummond et al. 2012), and divergence time estimates were made using an uncorrelated lognormal relaxed clock model (Drummond et al. 2006; Thorne et al. 1998; Thorne and Kishino 2002) and simple Yule model of speciation (Yule 1925; Gernhard et al. 2008).

BEAST uses a Bayesian MCMC model to estimate the topology, the substitution rates, and node ages. After successful preliminary runs with *ycf3*, three final runs of 10 million generations each were run on the CIPRES web portal (Miller et al. 2010). For estimates using ITS, convergence took longer so the three final runs ran 100 million generations each and burn-in was increased to twenty percent. We used Tracer v1.6 (Rambaut et al. 2014) to assess convergence between runs and to estimate mean and 95% highest posterior density (HPD) of age estimates based on the combined output. If effective sample size (ESS) for all parameters

exceeded 200 (as suggested by Drummond and Rambaut 2007) we considered the results reliable. Ten percent of our samples were discarded as burn-in and trees and parameter estimates from the three final runs were combined in LogCombiner v1.8.1 (Drummond et al. 2012). Posterior probabilities on interior nodes were summarized from the posterior using the program TreeAnnotator v1.8.1 (Drummond et al. 2012). Final figures were visualized in FigTree v1.4.2 (Rambaut 2012).

Due to the fleshy nature of most plants in Montiaceae, this family has a low fossilization potential and thus we were unable to find megafossils of Montiaceae in the literature. Instead we used a secondary calibration approach. Estimates from the Time Tree of Life (Hedges et al. 2006) provide a time estimate for the divergence between *Claytonia* and *Montia* at 20.4 million years ago (MYA). This estimate was used as a secondary constraint for analysis using *ycf3* sequences. These estimates result from work by Arakaki et al. (2011) and were determined based on comparison with other succulent lineages using 13 fossils as minimum-age node constraints. Arakaki et al. (2011) estimated the divergence time of the family Montiaceae to be 44.9-39.9 MYA. For estimates of divergence times made using ITS sequences we used a secondary constraint based on Ocampo and Columbus (2010). Using the biogeographic history of the Hawaiian islands and two endemic members of Portulacaceae, they estimated the most recent common ancestor (MRCA) of Montiaceae at 13 MYA. This family estimate was applied as a secondary constraint for divergence time analysis using ITS sequences.

RESULTS

Size and Structure of Individual Datasets – The final matrices used for analysis included 152 original sequences, but not all accessions were successfully amplified for all

markers. Detailed information on individual datasets is provided in Table 2.3. The aligned lengths of our nuclear datasets for ITS, *at103*, and *sqd1* were 536, 328, and 227 bp, respectively (Table 2.3). The aligned lengths of our plastid datasets for *trnK/matK*, *rps16*, *trnL-F* IGS, *trnS-trnG* IGS, and *ycf3-trnS* IGS were 1,315, 732, 408, 706, and 791 bp, respectively (Table 2.3). The combined *nr*DNA dataset was 1,091 bp in length, containing 127 variable sites (Table 2.3). Among the individual *nr*DNA partitions the percentage of informative sites varied from 2% for *sqd1* to 9% for ITS. The combined plastid DNA dataset was 3,952 bp in length and contained 378 variable sites. Among the individual *cp*DNA partitions the percentage of informative sites varied from 2% for the *ycf3-trnS* IGS to 6% for the *rps16* gene (Table 2.3)

Phylogenetic Analyses – Maximum likelihood analysis of all genetic markers supported (>85% ML BS) the monophyly of our sampled representatives of section *Rhizomatosae*. Our sampling of section *Claytonia*, represented by *C. eschscholtzii* and *C. tuberosa*, is also consistently supported in a distinct monophyletic clade sister to section *Rhizomatosae* (ML BS = 83–100%) by each of the individual markers (Fig. 2.1–2.8). However, resolution between lower level taxa is largely lacking and in some cases inconsistent.

Our results show slight incongruences between a number of the individual marker analyses for certain accessions or clades. In our analysis using the *cp*DNA *trnS-trnG* IGS (Fig. 2.7) *C. joanneana* (634, 636) appears in a supported clade (81% ML BS) with *C. arctica* (633), however, based on *nr*DNA ITS data (Fig 2.1) all accessions of *C. joanneana* and *C. sarmentosa* appear most closely related in a monophyletic and unambiguously supported clade (100% BS). Our analysis using the *cp*DNA *ycf3-trnS* IGS (Fig. 2.8) shows *C. noatakensis* (403) and *C. porsildii* (425) in an unambiguously supported clade (100% ML BS), whereas analysis based on the *cp*DNA *trnL-F* IGS (Fig. 2.6) shows *C. porsildii* (425) and *C. scammaniana* (007)

unambiguously supported (100% ML BS). Results from analysis of the *cp*DNA *rps16* (Fig. 2.5) show *C. porsildii* (426) in an unambiguously supported clade (100% ML BS) with *C. scammaniana* (011), while all other individual markers (Figs. 2.1–2.4; Fig. 2.8) show *C. porsildii* completely unresolved within a moderately to well supported clade of the remaining members of sect. *Rhizomatosae* sampled (85–100% ML BS).

The cladogram based on the full concatenated nuclear data (Fig. 2.9A) shows bootstrap support (82%) for a monophyletic sister group relationship between *C. joanneana* and *C. sarmentosa* with each species individually well to very well supported as monophyletic (90% ML BS and 99% ML BS, respectively). Analysis of the reduced concatenated nuclear dataset increased the support for the sister group relationship between *C. joanneana* and *C. sarmentosa* to 90% ML BS, but differed slightly in regards to the support for the monophyly of each of the species with 88% ML BS, and 100% ML BS respectively, but also unambiguously supported the monophyly of sect. *Claytonia* (ML BS 100%; Fig. 2.9B).

Analysis using BI showed similar topologies for all individual genetic markers using a 50% majority-rule consensus tree labeled with posterior probabilities (BI PP; Fig. 2.1–2.8). Although support values varied at the lower taxonomic levels, we again see the consistent monophyly of section *Rhizomatosae* (BI PP = .98 – 1.0) for all markers examined. Topologies were consistent with ML analyses except in three cases: a clade made up of three accessions of *C. noatakensis* (403, 435, 436) and one accession of *C. scammaniana* (011) that was weakly supported (75% ML BS) in ML analysis using the *at103* was not recovered in BI analysis. Our *trnK/matK* cladogram using BI lacks a *C. scammaniana* (438) and *C. joanneana* (635) clade that was weakly supported (63% ML BS) in ML analysis. Finally, BI analysis using the *trnS-trnG* IGS no longer supports *C. arctica* (633) in a clade with *C. joanneana* (634, 636), or *C.*

noatakensis (436) in a clade with *C. scammaniana* (010) and *C. sarmentosa* (009, 440) although both clades were weakly supported using ML (81% and 77% ML BS, respectively).

While MP analysis reflected the same general topology as ML and BI, parsimony bootstrap values (MP BS) were lower for almost all nodes in every marker analyzed and failed to show as much resolution within sect. *Rhizomatosae* as BI and ML analyses (Figs. 2.1–2.8). Parsimony analyses using *sqd1* (Fig. 2.3) and *trnK/matK* (Fig. 2.4) failed to complete likely due to the lack of variation between sequences and large portions of missing data, respectively. Parsimony analysis using *rps16* (Fig. 2.5) shows no support for relationships between *C. scammaniana* (011) and *C. porsildii* (426) or *C. arctica* (633) and *C. joanneana* (634). Similarly, parsimony analysis of the *trnS-trnG* IGS (Fig. 2.7) lacks support for a relationship between the two accessions of *C. joanneana* (634, 636) or for a clade containing *C. scammaniana* (010) and two accessions of *C. sarmentosa* (009, 440) as recovered with ML and BI analyses. All other topologies were congruent with ML and BI analyses.

Divergence Times Estimates in Montiaceae – Estimates of divergence times using ITS sequences are presented in Fig. 2.12. *Claytonia* was estimated to have diverged from its sister genus *Montia* at 8.98 MYA (95% HPD: 12.51–5.32 MYA) in the early Miocene (Table 2.4). Within *Claytonia*, the divergence of sect. *Limnia* from the clade comprised of sect. *Claytonia* and sect. *Rhizomatosae*, was inferred at 7.59 MYA (95% HPD: 11.05–4.48 MYA) in the late Miocene. The split of sect. *Rhizomatosae* (sensu O'Quinn and Hufford) from sect. *Claytonia* was inferred at 6.66 MYA (95 % HPD: 9.74 – 3.56 MYA), and the whole section shared a MRCA at 5.52 MYA (95% HPD: 8.62 – 2.74 MYA). Within sect. *Rhizomatosae,* Beringian members of the section are inferred to have shared a common ancestor at 3.20 MYA (95 % HPD: 5.28 – 1.30 MYA) with speciation having mostly occurred within the late Pleistocene.

Bayesian estimation of divergence in Montiaceae based on *ycf3* is presented in Fig. 2.13. Using *ycf3* sequences our estimate places *C. arenicola* in its own clade having diverged at 14.74 MYA (95% HPD 19.89–9.66 MYA) from the rest of the genus as opposed to being nested within sect. *Rhizomatosae*. Our estimates infer the divergence of sect. *Limnia* at 11.78 MYA (95% HPD: 16.25–7.40 MYA) from a clade comprised of sect. *Claytonia* and sect. *Rhizomatosae*. We also inferred the divergence of sect. *Rhizomatosae* including the non-Beringian *C. nevadensis* from sect. *Claytonia* at 9.62 MYA (95 % HPD: 14.15–5.73 MYA). The MRCA of sect. *Rhizomatosae* was inferred at 6.84 MYA (95% HPD 10.81–3.49 MYA) whereas the MRCA of Beringian members of sect. *Rhizomatosae* was estimated at 3.62 MYA (95 % HPD: 5.99–1.63 MYA).

DISCUSSION

Comparison of Phylogenetic Results – Our results provide additional molecular evidence from several markers to support the sectional divisions of *Claytonia* as circumscribed by O'Quinn and Hufford (2005), but the relationships between species present in Alaska remain unresolved (Figs. 2.1–2.10). Although all of our genetic markers are considered to be useful for resolving species level relationships in other plant groups (Palmer et al. 1988; Hamilton 1999; Shaw et al. 2005; Li et al. 2008), they offered little support for interspecific relationships within Beringian members of *Claytonia* sect. *Rhizomatosae* (Figs. 2.1–2.10). The marker most frequently used for molecular analyses in this group, the *nr*DNA ITS (Hershkovitz and Zimmer 2000; O'Quinn and Hufford 2005; Hershkovitz 2006a), was the most informative showing unambiguous clade support for *Claytonia* sect. *Claytonia*, sect. *Rhizomatosae* and a sister group relationship of *C. sarmentosa*/*C. joanneana* (Fig. 2.1). It also offered support (100% ML BS, BI

PP = .96) for the two accessions of *C. arctica* (631, 632) from near the Bering Strait in a monophyletic clade (Fig. 2.1). The only monophyletic and well supported results from our analysis were from our ITS and combined nuclear datasets (Fig. 2.1; Fig. 2.9).

Even within our well-supported clades there are incongruences between genetic markers. For example, *Claytonia* sect. *Claytonia*, represented by *C. tuberosa* and *C. eschscholtzii*, is supported by all individual markers (Figs. 2.1–2.8). However, neither of our full combination datasets shows high support for sect. *Claytonia* (Fig. 2.9A; Fig. 2.10), but phylogenetic analysis of the reduced combined nuclear dataset resulted in unambiguous support for both sect. *Claytonia* and sect. *Rhizomatosae* (Fig. 2.9B). Incongruences between individual trees may be responsible for the lack of additional support when markers are concatenated by genome (Fig. 2.9; Fig. 2.10), with some support values actually decreasing with concatenation (including support for the monophyly of sect. *Claytonia).*

This apparent conflict in results may very well be due to a disparity between species trees and gene trees (Pamilo and Nei 1988). We cannot assume that each of our individual markers followed the same evolutionary pathway. Conflicts between species and gene trees can be caused by genetic polymorphism, incomplete lineage sorting, allopolyploidy, and frequent hybridization (Maddison 1997; Page and Charleston 1997), all of which are frequently observed at high latitude (Johnson and Packer 1965; Abbott and Brochmann 2003; Leitch and Bennett 2004; Popp et al. 2005; Guggisberg et al. 2009). Our individual gene trees may reflect different relationships for certain accessions because the individual genes have different relationships than the species themselves. However, we found little sequence divergence that led to little resolution in any given gene tree, regardless of which marker was used. Therefore the lack of resolution in our

results appears to be for the most part due to a lack of sequence divergence rather than strongly conflicting gene trees.

The well-supported sister group relationship of *C. sarmentosa/ C. joanneana* – Our ITS ML and BI analysis shows unambiguous support (ML BS = 100%; BI PP = 1.0) for the monophyly of a *C. sarmentosa/C. joanneana* clade (Fig. 2.1). This relationship is also seen in our analysis using the full combined nuclear dataset (ML BS = 82%) and reduced combined nuclear dataset (ML BS = 90%). This sister group relationship is contrary to relationships proposed by the Pan Arctic Flora (PAF) which groups *C. joanneana* with *C. scammaniana* and *C. arctica* based on perennation structure and inflorescence similarities (Elven et al. 2011), but it corroborates Miller and Chambers (2006) assessment based on morphological similarities between *C. sarmentosa* and *C. joanneana* and O'Quinn and Hufford's (2005) molecular results from ITS data. Treatment in PAF recognizes similarities in perrenation structure citing a stout root stock and absence of stolons as a reason to group *C. joanneana* with *C. arctica* and *C. scammaniana*. Miller and Chambers (2006), on the other hand, cite leaf morphology and floral characters for grouping *C. joanneana* with *C. sarmentosa* and *C. arctica*. While similarities in perennation structures may be closely tied to sectional divisions in *Claytonia* (von Poellnitz 1932; Swanson 1966; O'Quinn 2005; O'Quinn and Hufford 2005; Jeffers 2015) it may not serve well as a delineator for species level relationships.

From our analyses of the concatenated nuclear dataset it appears that *C. sarmentosa*, which is found throughout Beringia and northwest North America, is most closely related to a Russian species, *C. joanneana*, as opposed to another Alaskan taxon (Fig. 2.1; Fig. 2.9). This is surprising considering that *C. sarmentosa* is much more prolific in Alaska and northwest North America, while *C. joanneana* is confined to western Beringia and mountainous regions of central

Asia (Miller and Chambers 2006). In addition, the two are believed to have survived Pleistocene glaciation in different refugia, *C. sarmentosa* in a southern Beringian refugium and *C. joanneana* in continental western Beringia (O'Quinn and Hufford 2005). The molecularly supported relationship between *C. sarmentosa* and *C. joanneana* echoes morphological similarities between the two morphological similarities of the leaves and flowers (Miller and Chambers 2006). Both *C. sarmentosa* and *C. joanneana* have typically spatulate leaves and white petals with pink veination.

Divergence and History – The Arctic flora is believed to be of relatively recent origin or representing relicts of Pleistocene (2.5–0.12 MYA) refugia (Hultén 1937; Murray 1995; Abbott and Brochmann 2003) and has been termed an "evolutionary freezer" by Brochmann et al. (2004). This is in large part due to dynamic and repeated changes since the last glacial maximum (0.26–0.2 MYA) (Fig. 2.11; Provan and Bennett 2008). Our divergence time estimations for *Claytonia* place the MRCA of Beringian members of sect. *Rhizomatosae* within the last 3.62 MYA, during the Pleiocene. This inferred age encompasses estimates made with both ITS and *ycf3,* at 3.20 MYA and 3.62 MYA respectively (Table 2.4). Our trees also show other sectional divergence time estimates are similar between the two analyses, but genus and family splits (Figs. 2.12–2.13) are significantly different (Table 2.4). This is due to the very different divergence time estimates for the family Montiaceae made by Ocampo and Columbus (2010) and Arakaki et al. (2011), at 39.9 MYA and 13 MYA respectively, upon which our secondary constraints were based. We consider the divergence estimates obtained from our analysis using *ycf3* sequences as more reliable due to the more widely accepted fossil dating that included 13 confidently placed fossil within a 90-taxon flowering plant phylogeny used by Arakaki et al. (2011). In contrast, the use of biogeographical history of the Hawaiian islands to infer age

constraints in Ocampo and Columbus (2010) provides a younger estimate, but the method for inferring divergence time is less traditionally accepted.

Occurrence data of *Claytonia* from ALA (Fig. 2.11) show a pattern of *Claytonia* currently concentrated in areas that were glaciated during the last glacial maximum (0.26 MYA–0.2M YA; Briner and Kaufman 2008; Kaufman et al. 2011). This would suggest that the majority of existing populations in Alaska have been established since that time either through establishment from nunatak populations preserved within the glaciated region or through migration from unglaciated Beringia to glacial margins as steppe turned to tundra (Edwards and Armbruster 1989; Bigelow 2003; Fedorov and Goropashnaya 2003). The recent divergence of Beringian *Claytonia* inferred at 3.6 MYA suggests that these two potential pathways have played a major role in the development of Beringian *Claytonia* diversification. Although many arctic-alpine species may be concentrated in areas that were previously glaciated (Abbott and Comes 2004; DeChaine et al. 2013), they may have arrived there in a variety of ways and generalizations about colonizations are ill-advised (Taberlet et al. 1998; Abbott and Brochmann 2003). Regardless, a more thorough sampling of this large portion of interior Alaska is needed for a better understanding of the biogeography, distribution, and phylogenetic relationships in Beringian *Claytonia*.

In conclusion, our results provide additional molecular support using several markers for previous sectional divisions within the genus *Claytonia* (O'Quinn and Hufford 2005). We also offer molecular evidence to support the sister species relationship between *C. joanneana* and *C. sarmentosa* in contrast to relationships proposed by PAF (Elven et al. 2011). However, this study fails to provide further resolution for most species level delineation despite using quickly evolving genetic markers. This lack of resolution is a reflection of recent divergence and likely

hybridization as opposed to slow rates of evolution. But in order to determine species level relationships a different approach is needed. Other studies of Beringian and arctic taxa have shown success with species level resolution using AFLPs and RFLPs (Stehlik et al. 2002; Ehrich et al. 2008; Westergaard et al. 2011). Advances in next generation sequencing (NGS) make a full estimate of sequence divergence possible (Emerson et al. 2010; Glenn 2011; Egan et al. 2012) and could hold promise to resolving species level relationships in *Claytonia* sect. *Rhizomatosae*. Molecular phylogenetic studies are probably not going to recover sufficient data to resolve species level relationships within *Claytonia* section *Rhizomatosae*. Rather a population level approach may be able to address these questions.

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TABLE 2.1. Primers used for PCR amplification and sequencing. Individual loci, primers, associated sequences, genomic compartment, and references are listed below. For loci with only two primers the same pair were used for both amplification and sequencing. For *matK/trnK* multiple iterations of forward primer and reverse primer were used in PCR amplification if any individual accession had trouble sequencing.

Locus	Primer	Sequence 3'–5'	Genome	References
ITS	*Nnc18S10*	AGGAGAAGTCGTAACAAG	Nuclear	Soltis et al. (1997)
	C26A	TTTCTTTTCCTCCGCT		
at103	*at103F*	CTTCAAGCCMAAGTTCATCTTCTA	Nuclear	Rzeznicka et al.
	at103R	TTGGCAATCATTGAGGTACATNGTMACATA		(2005)
sqd1	*sqd1F*	CTTGGGACSATGGGTGARTATGG	Nuclear	Essigmann et al.
	sqd1R	CCWACAGCAGCYTGMACACAGAACC		(1999)
*mat*K/ *trn*K	*matK–*	AGGATGTTGATYGTAAATGA	Plastid	Johnson and Soltis
	1470R			(1994)
	trnK–	TGGGTTGCTAACTCAATGG		
	3914F			
	360F	CGGGAAAGGCTTCTCCCACG		O'Quinn and
	670R	GGAATTTCCACAATGACTGC		Hufford (2005)
rps16	*rps16–F*	GTGGTAGAAAGCAACGTGCGACTT	Plastid	Oxelman et al.
	rps16–R2	TCGGGATCGAACATCAATTGCAAC		(1997)
trnL–F	*trnLc*	CGAAATCGGTAGACGCTACG	Plastid	Taberlet et al. (1991)
	trnLd	GGGGATAGAGGGACTTGAAC		
trnS–G	*trnS*	GCCGCTTTAGTCCACTCAGC	Plastid	Hamilton (1999)
	trnG	GAACGAATCACACTTTTACCAC		
ycf3–trnS	*SP43095F*	TTTCTCCTGAAGTTGTCGGAAT	Plastid	Hershkovitz &
	SP43097R	ATTCGAACCCTCGGTAAACA		Zimmer (2006)

TABLE 2.2. Thermocycler conditions used for PCR amplification. Individual marker protocols for thermocycler conditions are listed. After initial denaturation ITS used a touchdown method of five cycles of 1 min at 94°C, 1 min at 53°C (decreasing by 1°C each cycle to 48°C), and 2 min at 72°C before proceeding to the next step.

Marker	Initial denature	x35 cycles			Final extension
		Denature	Anneal	Extend	
ITS	4 min at 95°C	1 min at 94°C	1 min at 48°C	2 min at 72°C	5 min at 72°C
at103	2 s at 95°C	40 s at 94°C	30 s at 51°C	40 s at 72°C	5 min at 72°C
sqd1	2 s at 95°C	40 s at 94°C	30 s at 54.5°C	40 s at 72°C	5 min at 72°C
trnK/matK	3 min at 94°C	1 min at 94°C	1 min at 48°C	3 min at 72°C	15 min at 72°C
rps16	5 min at 95°C	45 s at 95°C	1 min at 53°C	1 min at 72°C	7 min at 72°C
trnL–F	None	1 min at94°C	1 min at 55°C	3 min at 72°C	None
trnS–G	5 min at 80°C	1 min at 95°C	50°C + 0.3°C/s to 65°C for 1 min	5 min at 65°C	65°C for 10 min
ycf3-trnS	2 min at 93°C	1 min at 93°C	1 min 20 s at 55°C	1 min 30 s at 72°C	5 min 30 s at 72°C

TABLE 2.3. Characteristics of individual *nr*DNA, *cp*DNA and combined datasets and resulting trees. Detailed information given is for individual datasets. For concatenated datasets, only maximum likelihood analysis was performed. Model selection for concatenated maximum likelihood analysis was partitioned based individual model selection for the individual markers.

	nrDNA regions				cpDNA regions				Concatenated	
	ITS	*at103*	*sqd1*	*rps16*	*trnL–trnF* IGS	*trnS–trnG* IGS	*ycf3 –trnS* IGS	*matK*	nuclear	plastid
Aligned lengths (bp)	536	328	227	732	408	706	791	1315	1091	3952
Number of characters included	532	328	227	722	408	580	727	1251	1087	3688
Number of variable sites	59	36	19	59	17	54	54	307	127	378
Total number of parsimony	49	14	5	44	17	24	16	178	N/A	N/A
informative characters (%)	(9%)	(4%)	(2%)	(6%)	(4%)	(4%)	(2%)	(14%)		
Number of taxa included	24	16	18	22	11	25	15	21	27	27
(OG/*Claytonia*)	(2/22)	(1/15)	(1/17)	(2/20)	(–/11)	(2/23)	(1/14)	(2/19)	(2/25)	(2/25)
Number of clades with MLBS>80%,	8, 6, 8	3, 2, 4	2, –, 3	6, 3, 6	3, 3, 3	8, 4, 6	5, 3, 6	5, –, 4	7	4
MPBS>80%, and BIPP>.9										
Model selected under AIC	GTR+G	HKY	K80	GTR+I	F81	HKY+I	GTR+G	GTR+G	a priori	a priori

TABLE 2.4. Inferred estimates of node ages for ITS and *ycf3* sequences. Estimates of most recent common ancestor ages (MRCA, crown ages) are shown below with associated 95% highest posterior density (HPD) intervals. *The node representing MRCA for Montiaceae using *ycf3* sequences is not shown in our cladogram, but was inferred in our analysis using the Arakaki et al. (2011) estimate which placed the crown age of Montiaceae at 39.9 MYA +/- 3.1 standard deviations. **The *ycf3* estimate for the MRCA age of sect. *Rhizomatosae* does not include *C. arenicola*, which is the first diverging clade using when using *ycf3* sequences for analysis.

Estimates using ITS

MRCA	Clade	Age (MYA)	95% HPD (MYA)
1	Montiaceae	13.32	8.03 –18.42
2	*Montia + Claytonia*	8.98	5.32 – 12.51
3	*Claytonia*	7.59	4.48 – 11.05
4	*Rhizomatosae*	5.52	2.74 – 8.62
5	Beringian *Rhizomatosae*	3.20	1.30 – 5.28

Estimates using *ycf3*

MRCA	Clade	Age (MYA)	95% HPD (MYA)
1*	Montiaceae	39.9	See note
2	*Montia + Claytonia*	20.4	15.92 – 25.06
3	*Claytonia*	14.74	9.66 – 19.89
4	*Rhizomatosae***	6.84	3.49 – 10.81
5	Beringian *Rhizomatosae*	3.62	1.63 – 5.99

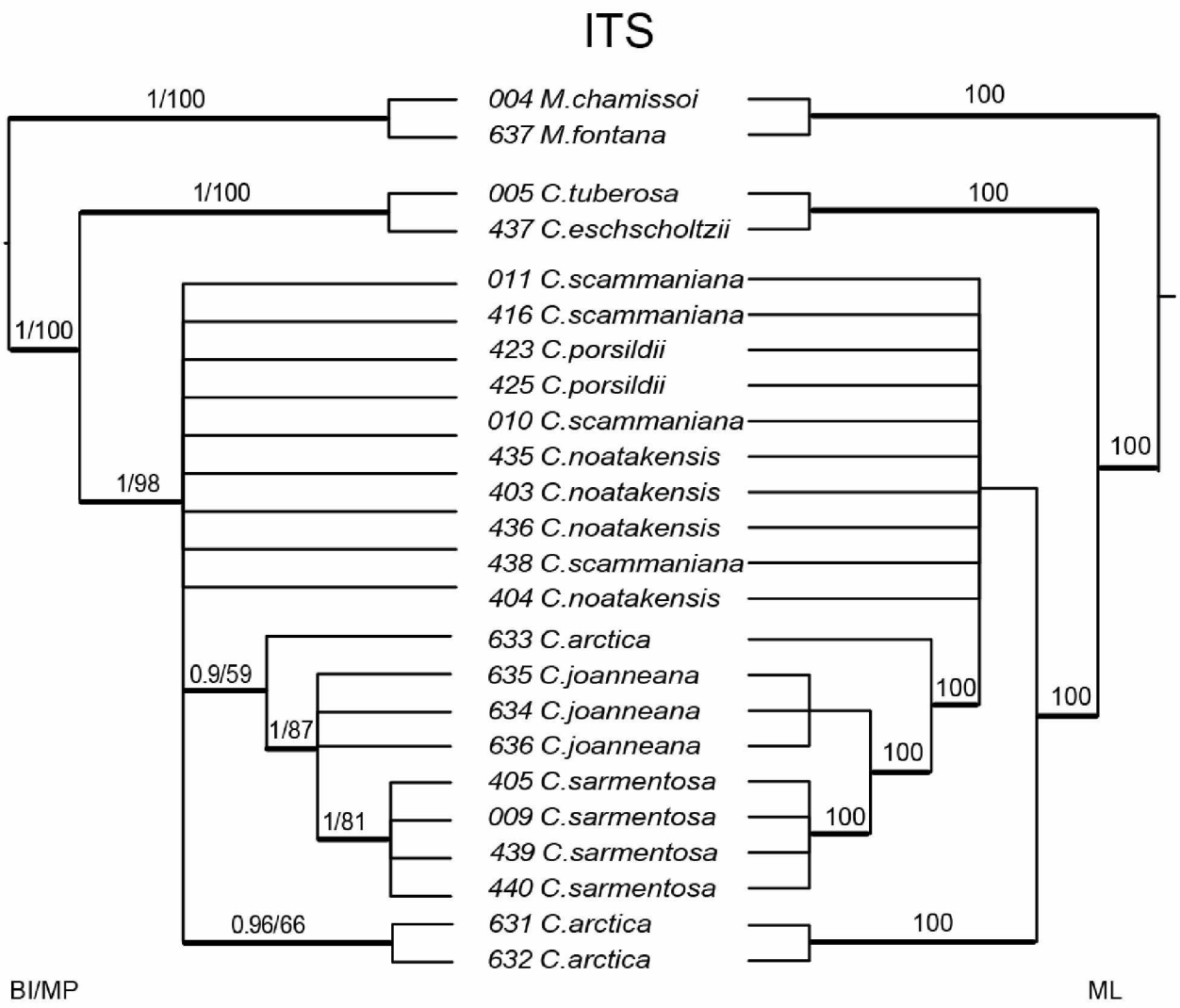

FIGURE 2.1. Comparison of different phylogenetic inference methods on tree topology using nuclear ribosomal ITS. Cladogram showing phylogenetic relationships using ITS sequences based on maximum likelihood (ML) at right, and maximum parsimony (MP) and Bayesian inference (BI) shown at left. Support values indicated are BI PP followed by MP BS for BI/MP analyses on the left, while ML BS is indicated for ML analysis above branches on the right. Bold branches have > 80 % BS support or > .8 PP.

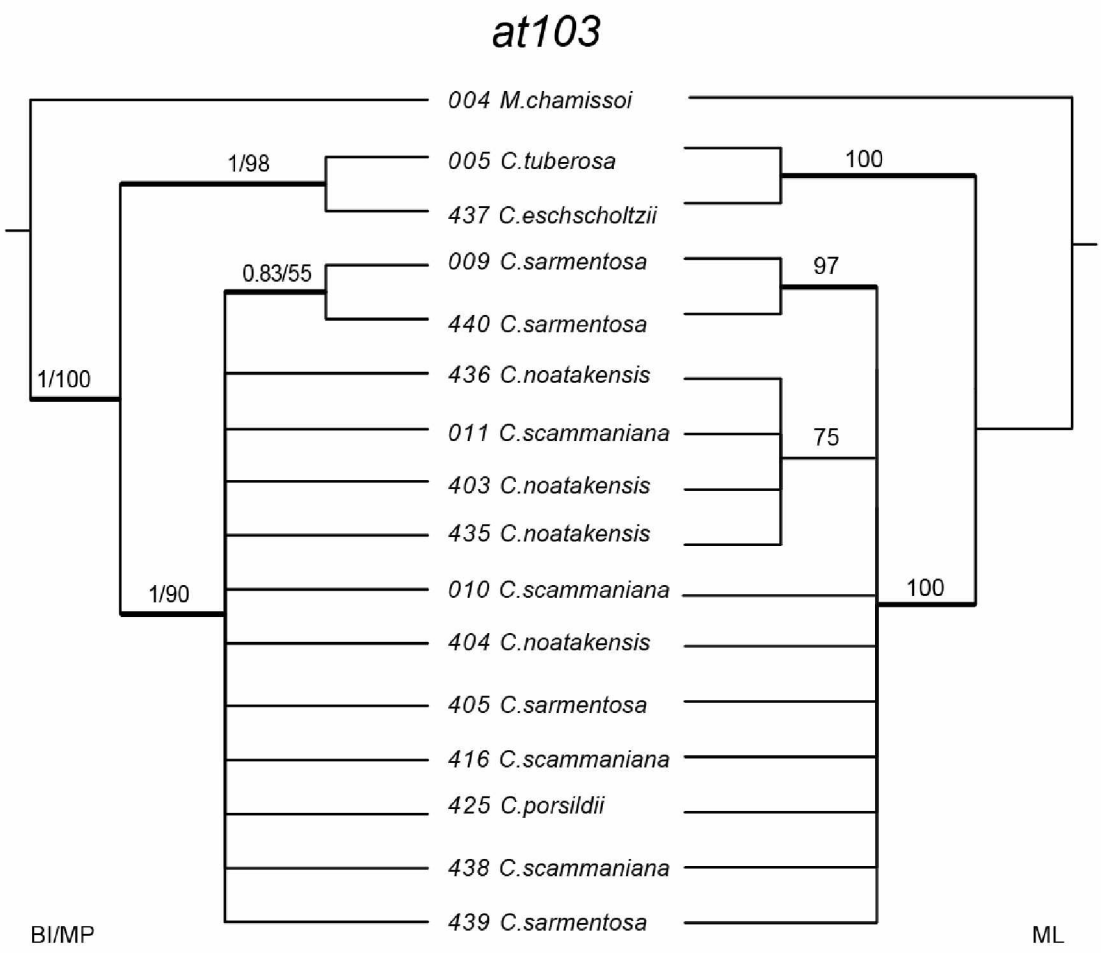

FIGURE 2.2. Comparison of different phylogenetic inference methods on tree topology using nuclear *at103*. Cladogram showing phylogenetic relationships using *at103* sequences based on maximum likelihood (ML) at right, and maximum parsimony (MP) and Bayesian inference (BI) shown at left. Support values indicated are BI PP followed by MP BS for BI/MP analyses on the left, while ML BS is indicated for ML analysis above branches on the right. Bold branches have > 80 % BS support or > .8 BI PP.

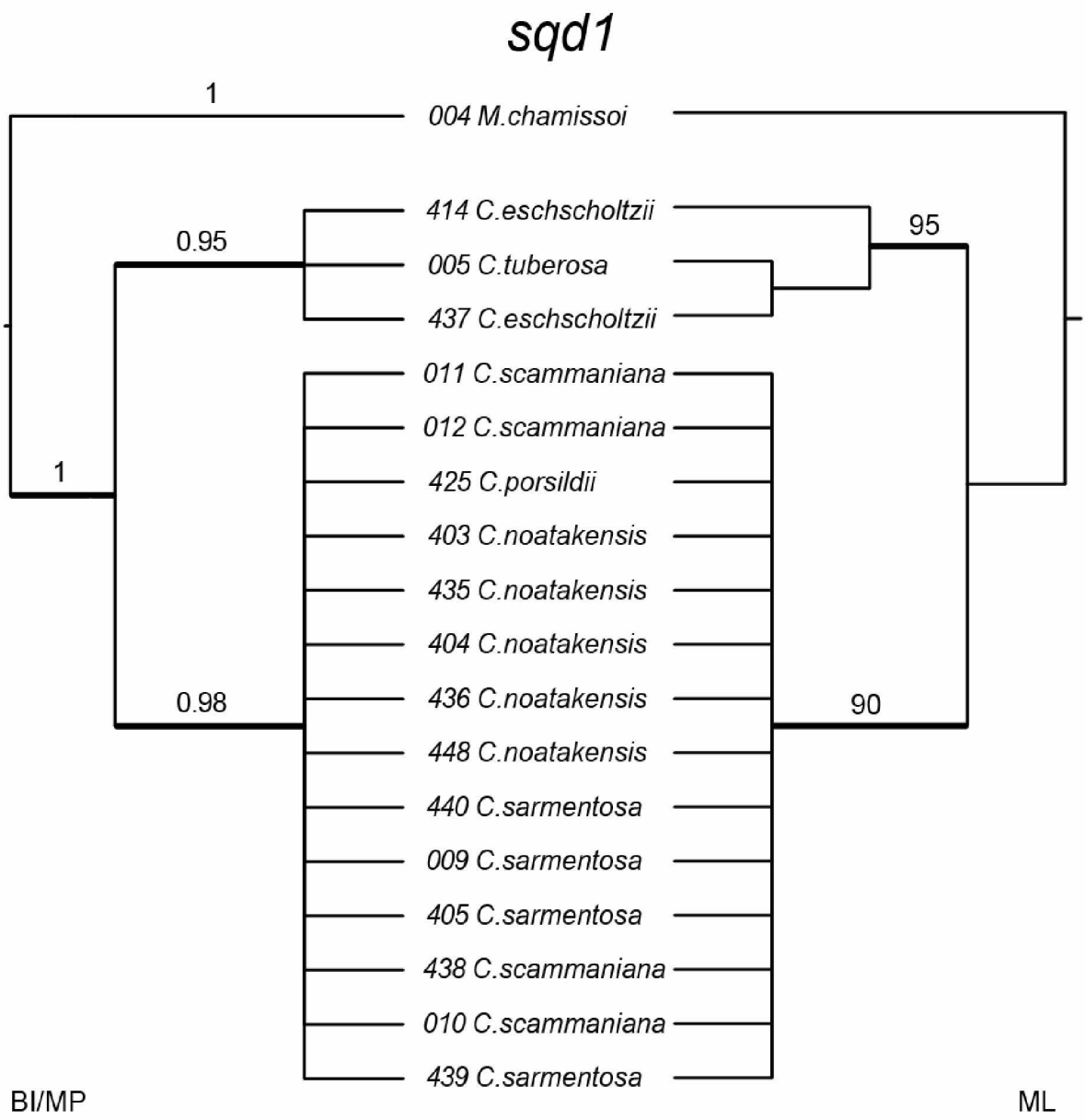

FIGURE 2.3. Comparison of different phylogenetic inference methods on tree topology using nuclear *sqd1*. Dendrogram showing phylogenetic relationships using *sqd1* sequences based on maximum likelihood (ML) at right, and maximum parsimony (MP) and Bayesian inference (BI) shown at left. Support values indicated are BI PP followed by MP BS for BI/MP analyses on the left, while ML BS is indicated for ML analysis above branches on the right. Bold branches have > 80 % BS support or > .8 PP.

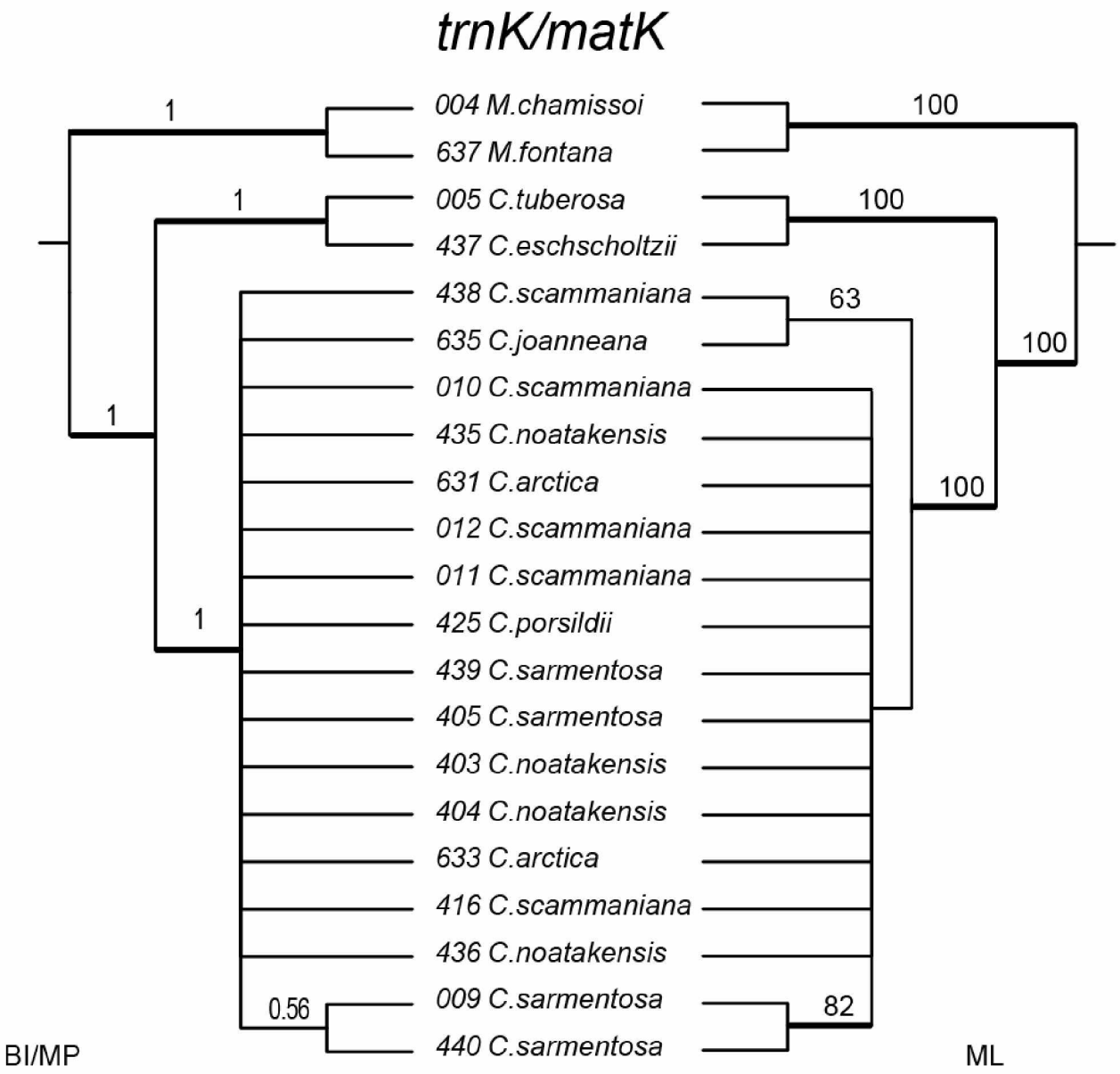

FIGURE 2.4. Comparison of different phylogenetic inference methods on tree topology using plastid *trnK/matK*. Cladogram showing phylogenetic relationships using *trnK/matK* sequences based on maximum likelihood (ML) at right, and maximum parsimony (MP) and Bayesian inference (BI) shown at left. Support values indicated are BI PP followed by MP BS for BI/MP analyses on the left, while ML BS is indicated for ML analysis above branches on the right. Bold branches have > 80 % BS support or > .8 PP.

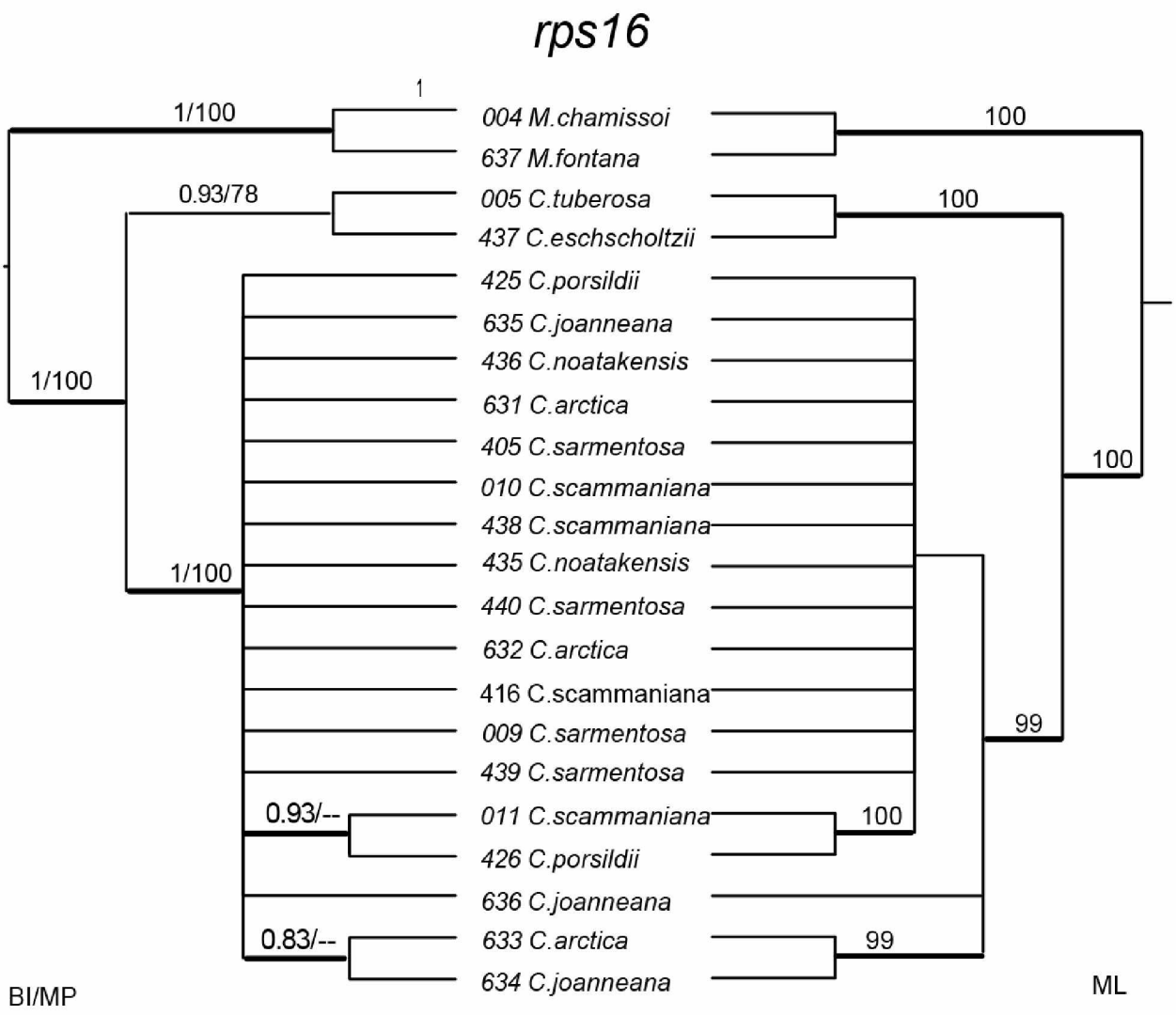

FIGURE 2.5. Comparison of different phylogenetic inference methods on tree topology using plastid *rps16*. Cladogram showing phylogenetic relationships using *rps16* sequences based on maximum likelihood (ML) at right, and maximum parsimony (MP) and Bayesian inference (BI) for *rps16* shown at left. Support values indicated are BI PP followed by MP BS for BI/MP analyses on the left, while ML BS is indicated for ML analysis above branches on the right. Bold branches have > 80 % BS support or > .8 PP.

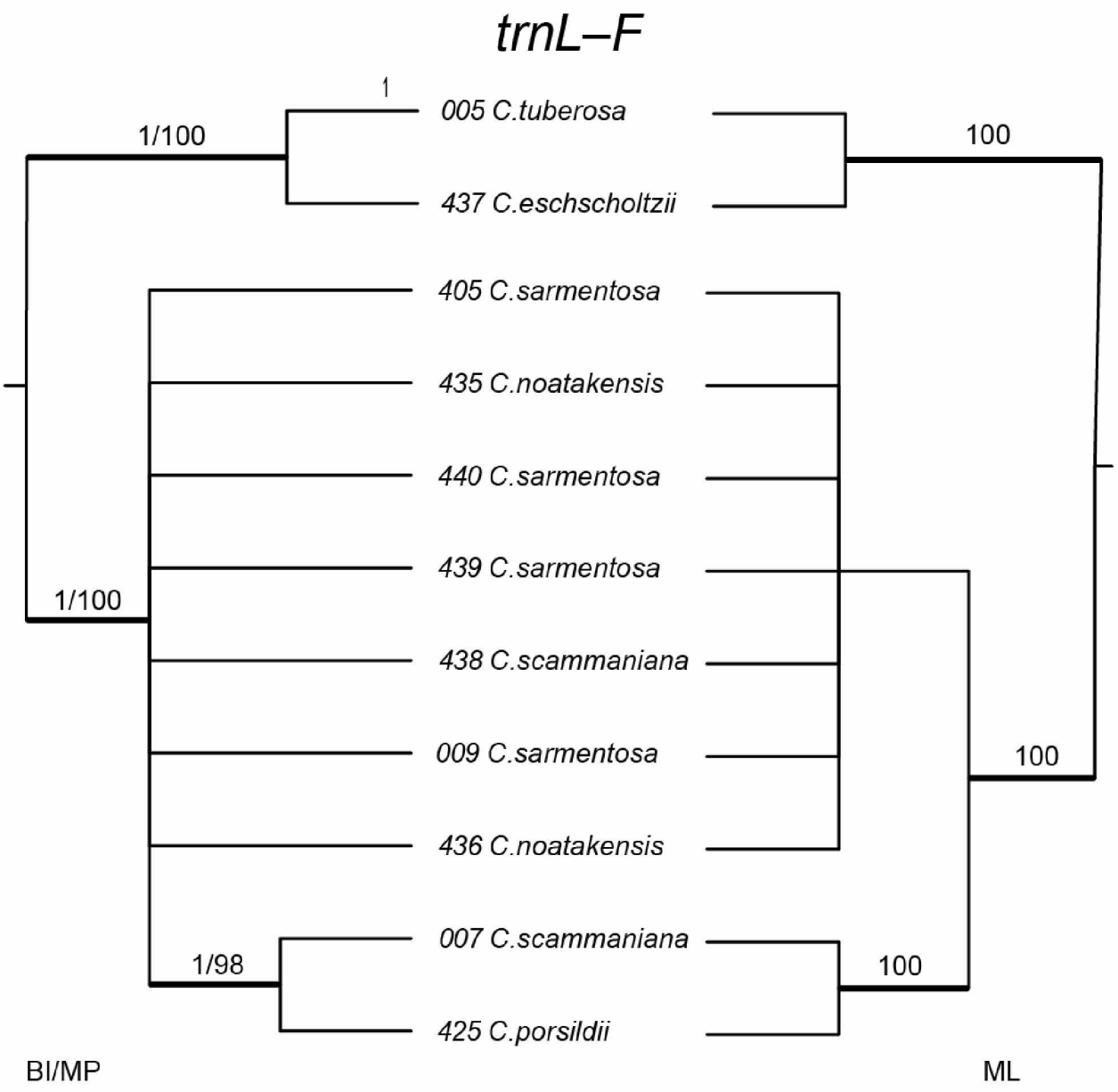

FIGURE 2.6. Comparison of different phylogenetic inference methods on tree topology using plastid *trnL-F*. Cladogram showing phylogenetic relationships using *trnL-F* sequences based on maximum likelihood (ML) at right, and maximum parsimony (MP) and Bayesian inference (BI) for *trnL-F* shown at left. Support values indicated are BI PP followed by MP BS for BI/MP analyses on the left, while ML BS is indicated for ML analysis above branches on the right. Bold branches have > 80 % BS support or > .8 PP.

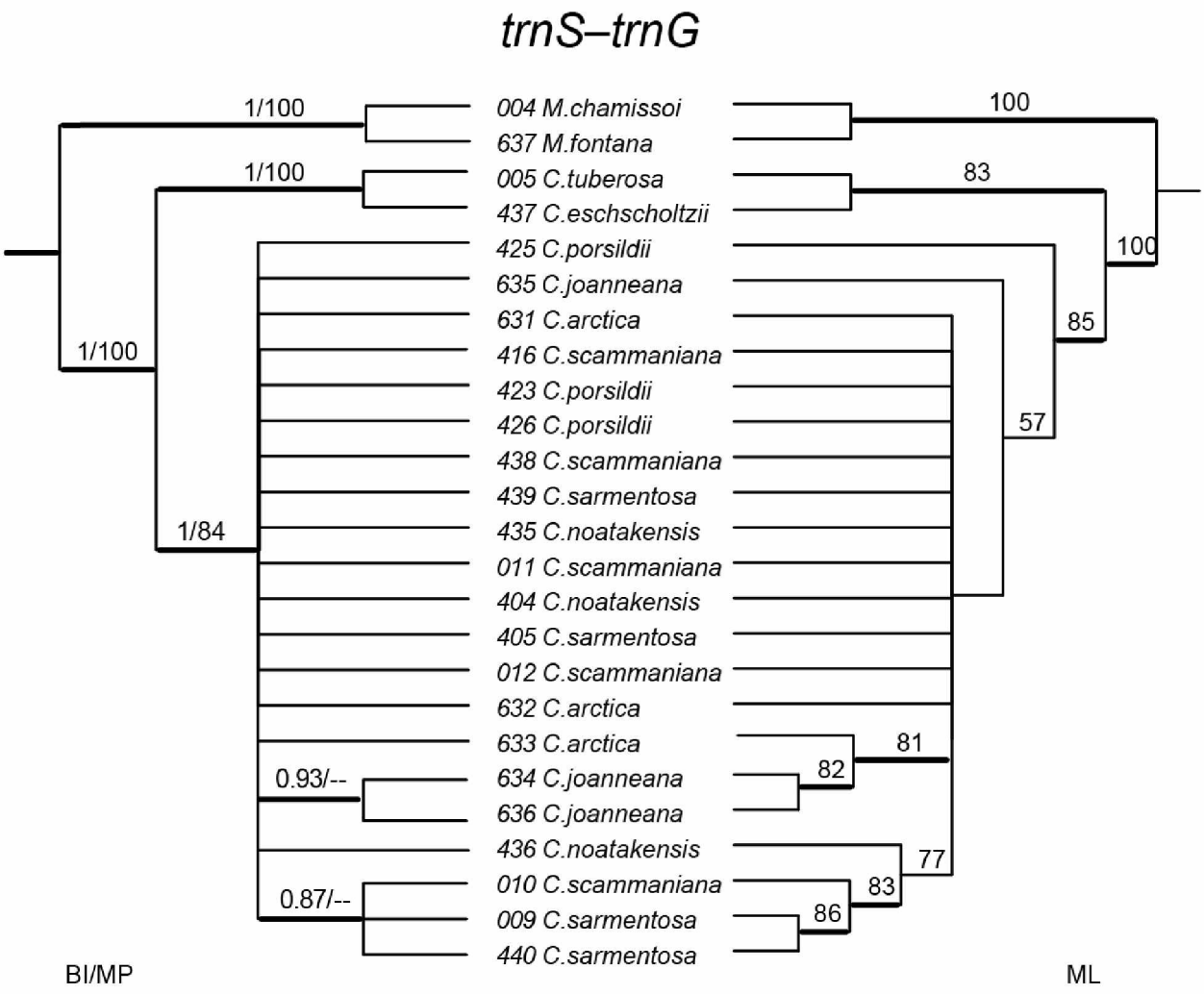

FIGURE 2.7. Comparison of different phylogenetic inference methods on tree topology using plastid *trnS-G*. Cladogram showing phylogenetic relationships using *trnS-G* sequences based on maximum likelihood (ML) at right, and maximum parsimony (MP) and Bayesian inference (BI) for *trnS-G* shown at left. Support values indicated are BI PP followed by MP BS for BI/MP analyses on the left, while ML BS is indicated for ML analysis above branches on the right. Bold branches have > 80 % BS support or > .8 PP.

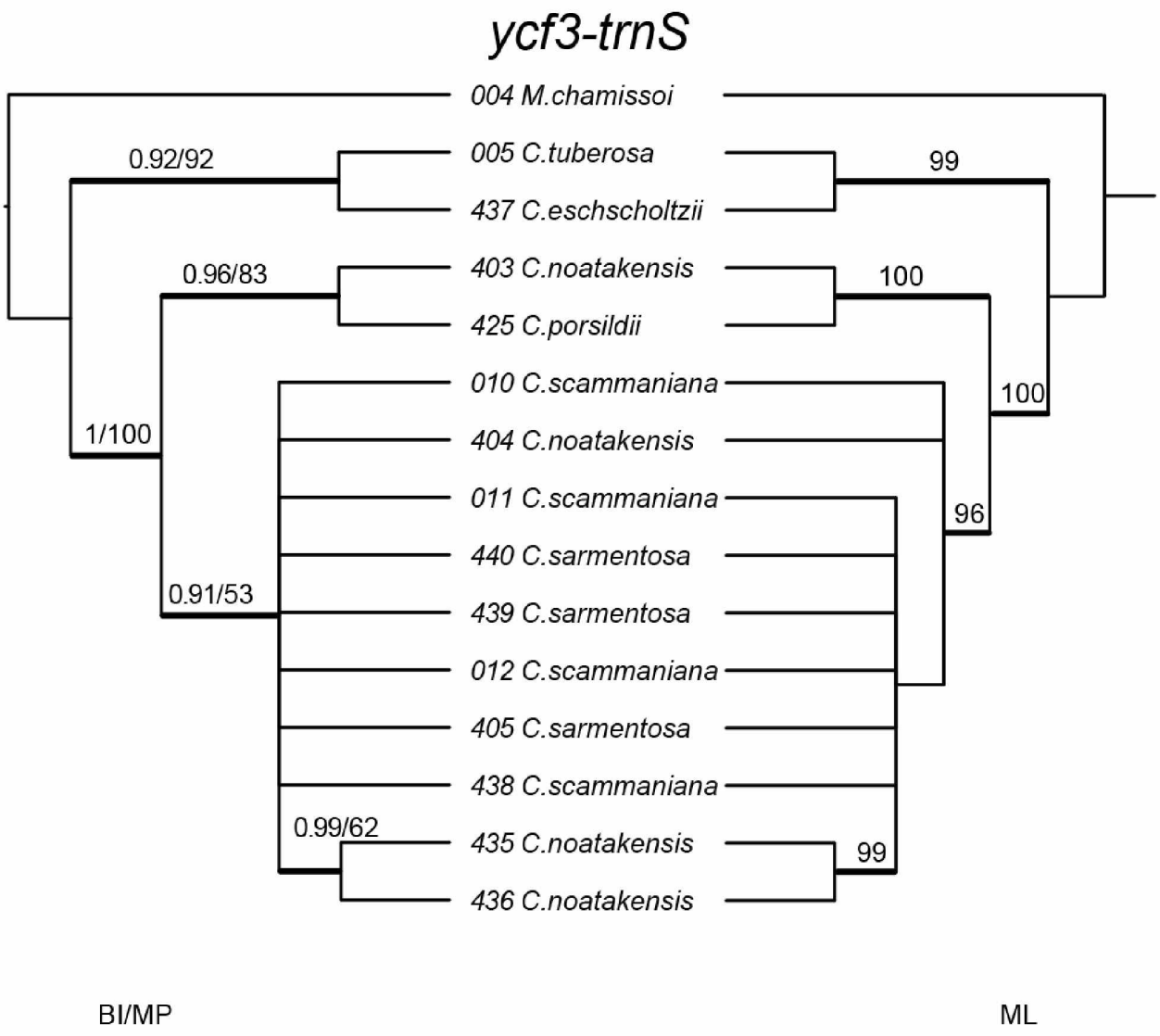

FIGURE 2.8. Comparison of different phylogenetic inference methods on tree topology using plastid *ycf3-trnS*. Cladogram showing phylogenetic relationships using *ycf3-trnS* sequences based on maximum likelihood (ML) at right, and maximum parsimony (MP) and Bayesian inference (BI) for *ycf3-trnS* shown at left. Support values indicated are BI PP followed by MP BS for BI/MP analyses on the left, while ML BS is indicated for ML analysis above branches on the right. Bold branches have > 80 % BS support or > .8 PP.

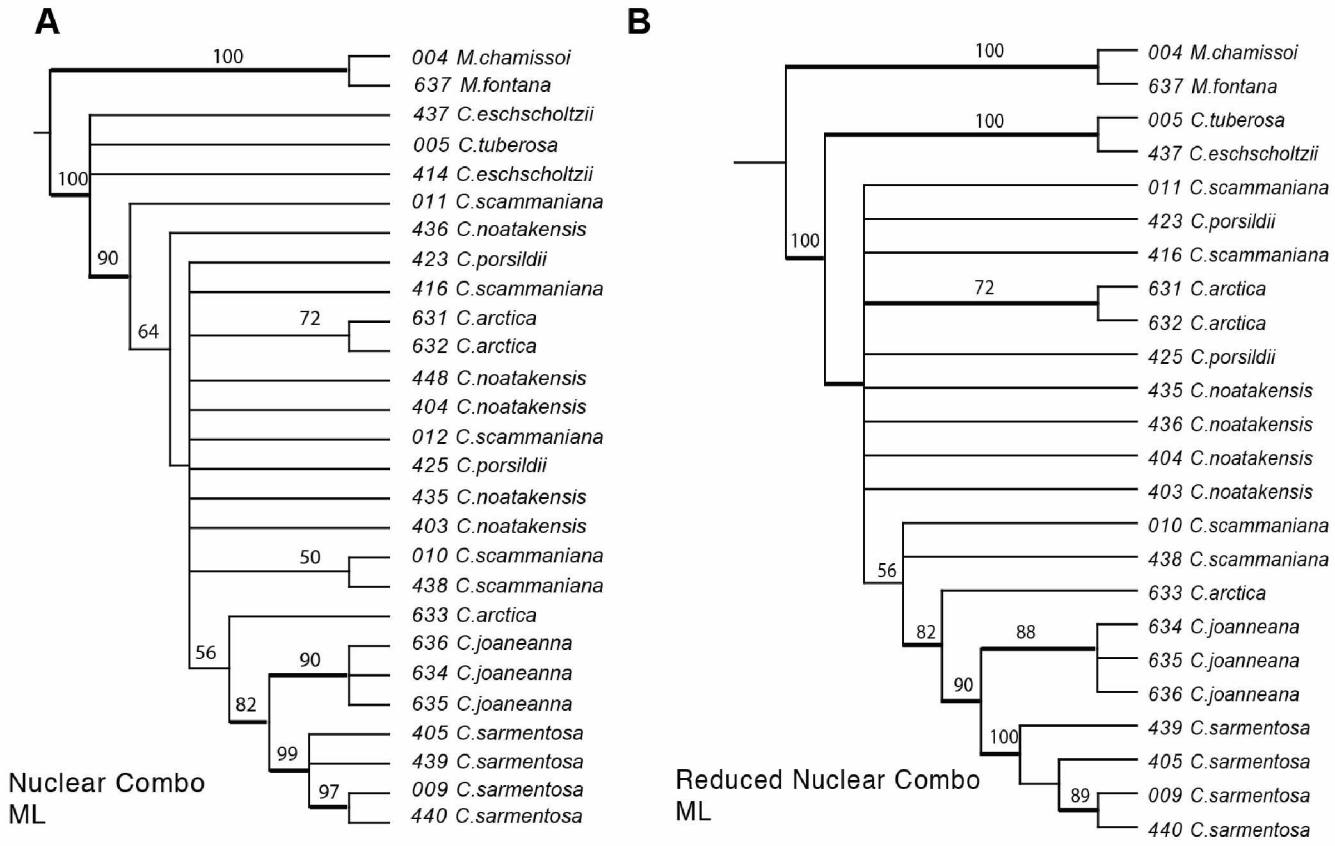

FIGURE 2.9. Comparison of tree topology inferred using ML based on full concatenated versus a reduced concatenated nuclear dataset. A. Full combined nuclear dataset. B. Reduced combined nuclear dataset. These cladograms show phylogenetic relationships using both the full combined nuclear dataset on the left and the reduced nuclear dataset on the right based on maximum likelihood analysis. The reduced dataset removes accessions lacking sequences for ITS. ML BS is indicated above branches. Bold branches have > 80 % ML BS support.

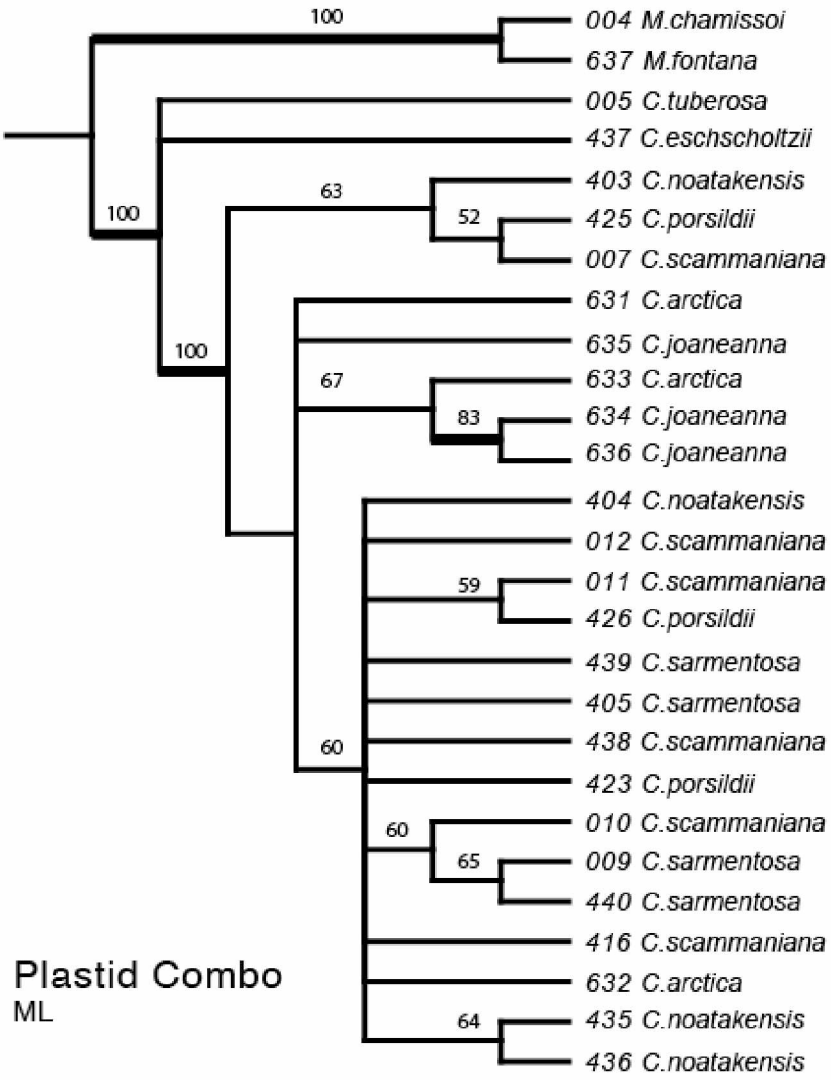

FIGURE 2.10. Phylogenetic relationships using the combined plastid dataset. Cladogram showing phylogenetic relationships using the combined plastid dataset based on maximum likelihood (ML) analysis. ML BS is indicated above branches on the right. Bold branches have >80 % ML BS support.

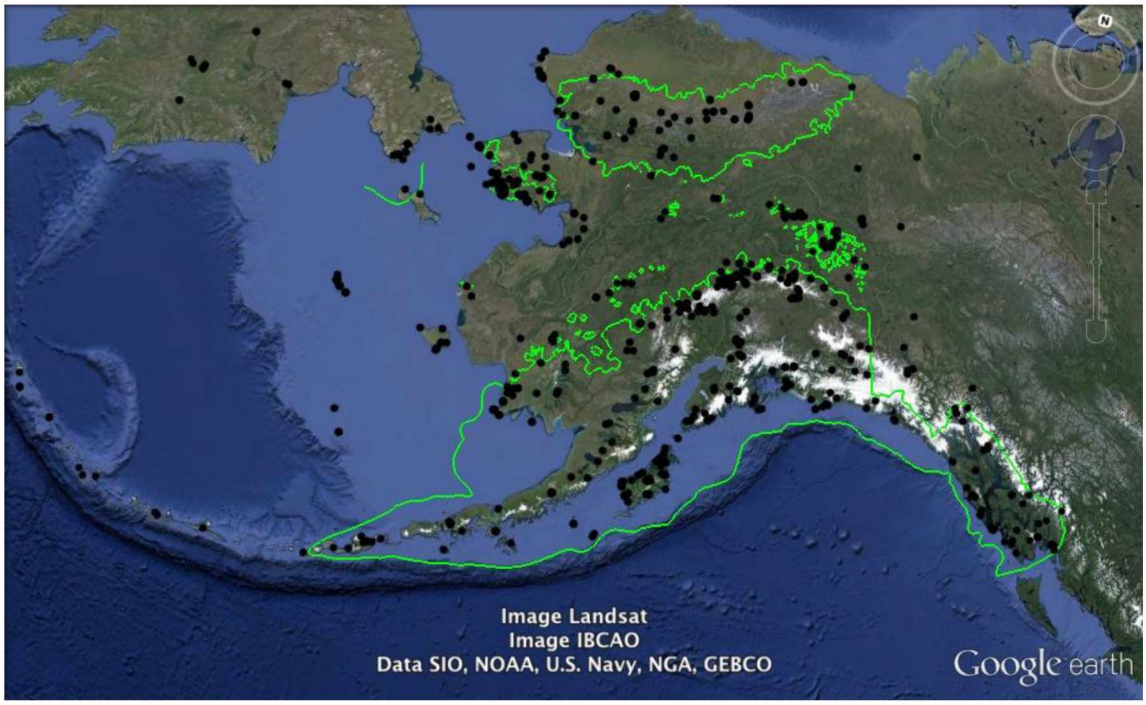

FIG. 2.11. Physical map of Beringian *Claytonia* occurrence data and outline of the extent of the last glacial maximum (0.20 – 0.26 MYA) in Alaska. This map shows all occurrence data of *Claytonia* present at ALA. The green outline represents ice coverage during the last glacial maximum (US only) based on the Alaska Palaeo-Glacier Atlas (Version 2) from the NOAA National Climatic Data Center (Kaufman et al. 2011).

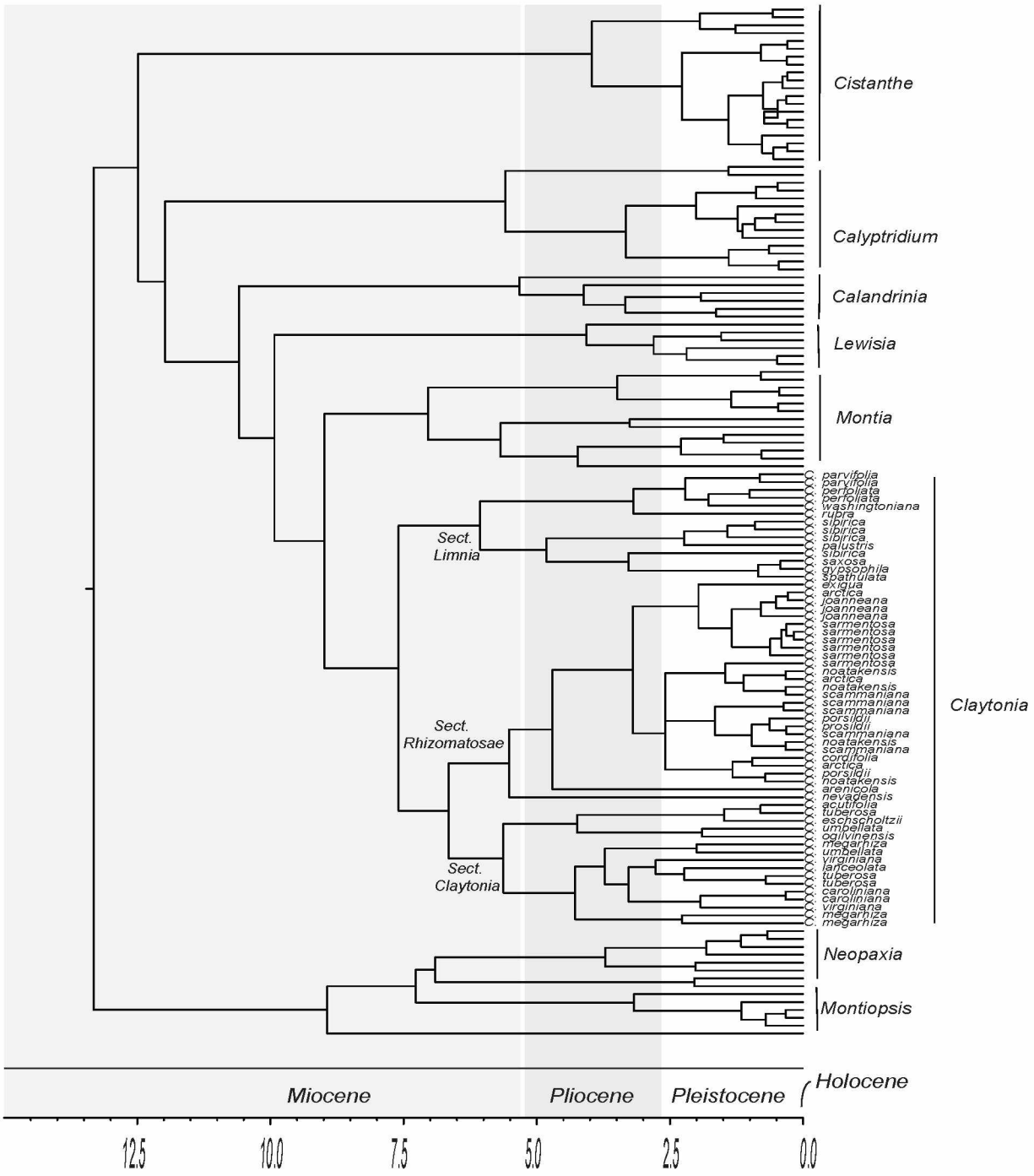

FIG. 2.12. Chronogram of Montiaceae divergence times using ITS sequences. The tree above shows estimated time of divergence (in MYA) for members of Montiaceae based on analysis using ITS sequences. Genera are indicated at the right. A number on the chronogram depicts nodes of particular relevance to our study and inferred ages are shown in the legend with HPD intervals in parentheses.

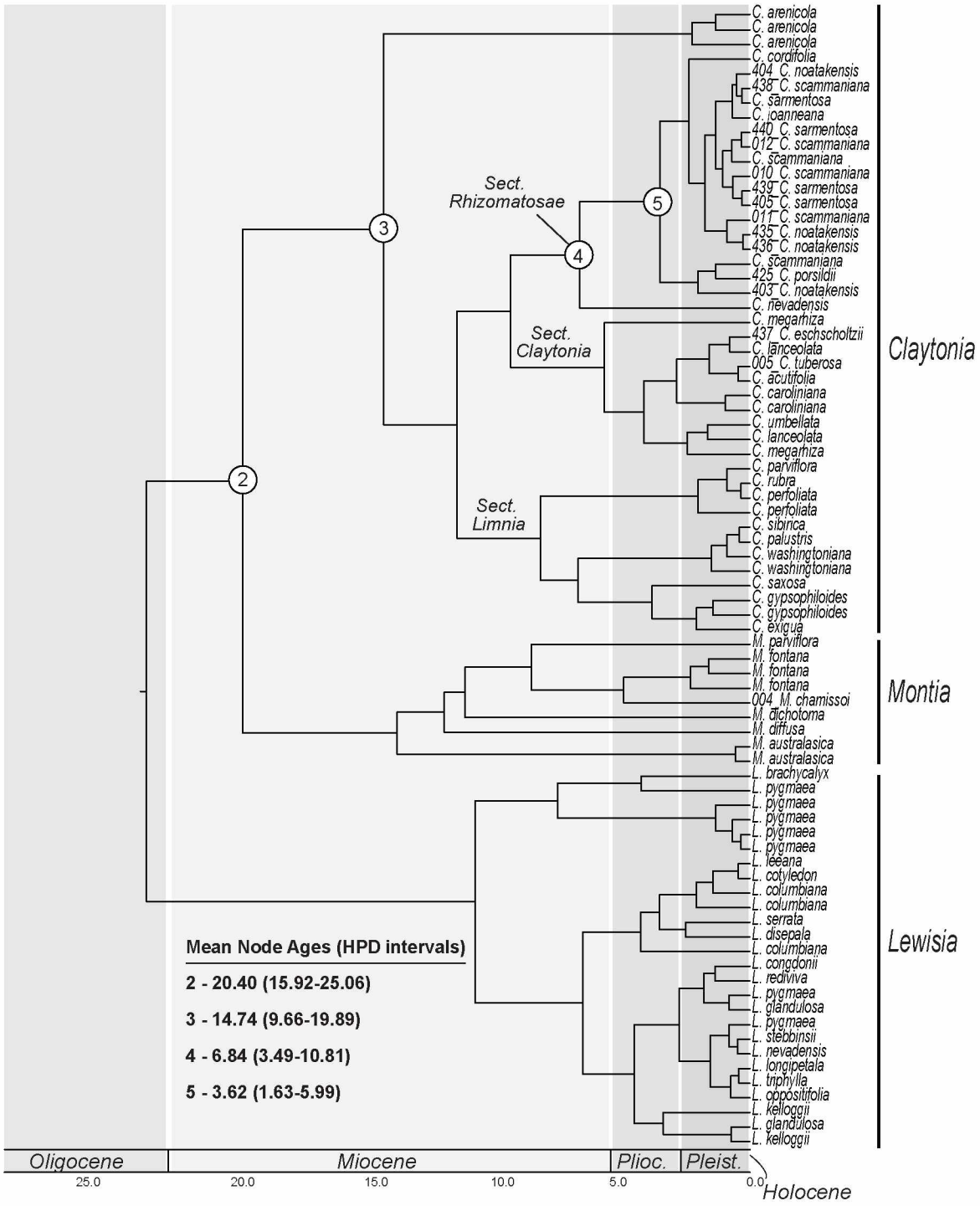

FIG. 2.13. Chronogram of *Claytonia* divergence times using *ycf3* sequences. This figure depicts the divergence time estimates (in MYA) made using *ycf3* sequences. Genera are indicated at right. Nodes of particular relevance to our study are depicted by a number on the chronogram and inferred ages are shown in the legend with HPD intervals in parentheses.

APPENDIX 2.1. Voucher information with locality information and ALA accession numbers. The listed specimens were used for DNA extraction. They are listed with scientific name, collector(s), collector number, locality, and either the herbarium accession number or notation of live collection. Extractions labeled live collection were made from fresh material that was collected by Stephany Jeffers. Some of these live collections were brought back for greenhouse observation. Upon completion of study all materials will be pressed, cataloged and voucher specimens will be annotated.

INGROUP:

***Claytonia arctica* Adams**

631 *C. arctica* (R. Lipkin 96-21, Seward Peninsula, AK) V143814; 632 *C. arctica* (C. Xapkebna & M. Ropma 28.06.1975, Kamchatka, Russia) V110028; 633 *C. arctica* (H. Solstad & R. Elven 04/0480, Chekurovka, Sakha Republic,Yakutia, Russia) V154330.

***Claytonia eschscholtzii* Cham.**

414 *C. eschscholtzii* (C. L. Parker, R. Lipkin, & C. Meyers 15976, Seward Peninsula, AK) V155035; 437 *C. eschscholtzii* (S. Jeffers 101, Feniak Lake, Noatak National Preserve, AK) Live Collection.

***Claytonia joanneana* Roem. & Schult**

634 *C. joanneana* (M. B. Sokolova, L. M. Zudova s.n, Taymyr Autonomous Okrug, Russia) V86648; 635 *C. joanneana* (T. Elias, S. Shetler, D. Murray 7325, Altai Mountains, Gorno-Altayskaya autonomous oblast, Russia) V80470; 636 *C. joanneana* (N. V. Matveyeva s.n., Taimyr, Pyasin, Russia) V68816.

***"Claytonia noatakensis"* (Young 1974)**

Collection; 404 *"C. noatakensis"* (C. L. Parker 15615, Goodnews Bay Quad, AK) V150049 *filed as *C. scammaniana;* 435 *C. noatakensis* (S. Jeffers 103, Feniak Lake, Noatak National Preserve, AK) Live Collection; 436 *C. noatakensis* (S. Jeffers 104, Feniak Lake, Noatak National Preserve, AK) Live Collection; 448 *C. noatakensis* (S. Jeffers 105, Feniak Lake, Noatak National Preserve, AK) Live Collection.

***Claytonia porsildii* Jurtzev.**

423 *C. porsildii* (C. L. Parker, H. Solstad 13433, Chandler Lake Quad, Gates of the Arctic National Park and Preserve, AK) V139770 *filed as *C. scammaniana*; 425 *C. porsildii* (M. Duffy MD02-45, Talkeetna Quad, Denali National Park and Preserve) V148793* filed as *C. scammaniana*.

***Claytonia sarmentosa* C. A. Mey.**

009 *C. sarmentosa* (S. Jeffers 106, Eagle Summit, AK) Live Collection; 405 *C. sarmentosa* (S. Jeffers 107, Hatcher's Pass, AK) Live Collection; 439 *C. sarmentosa* (S. Jeffers 108, Hatcher's Pass, AK) Live Collection; 440 *C. sarmentosa* (S. Jeffers 109, Feniak Lake, Noatak National Preserve, AK) Live Collection.

***Claytonia scammaniana* Hultén**

007 *C. scammaniana* (A. Larsen, A. R. Batten 01-747, Alaska Range, headwaters of Dillinger R. at W end Shellabarger Pass, AK) V138801; 011 *C. scammaniana* (C. L. Parker, H. Solstad 13434, Chandler Lake Quad, Gates of the Arctic National Park and Preserve, AK) V139771; 012 *C. scammaniana* (C. Roland 3198, Healy Quad, Denali National Park and Preserve, AK) V127384; 416 *C. scammaniana* (C. L. Parker & C.R. Meyers 10592, Noatak National Preserve, Howard Pass Quad, AK) V134540; 438 *C. scammaniana* (S. Jeffers 110, Eagle Summit, AK) Live Collection.

***Claytonia tuberosa* Pall. ex. Willd.**

005 *C. tuberosa* (S. Jeffers 111, 12 Mile Summit, AK) Live Collection.

OUTGROUP:

***Montia chamissoi* Tidestr.**

004 *M. chamissoi* (S. Studebaker 09-305, Karluk Quad, Kodiak Island, AK) V167863.

***Montia fontana* L.**

637 *M. fontana* (C. L. Parker 15903 Kuskokwim Bay, Goodnews Bay, AK) V150412.

CONCLUSION

Despite its charismatic appearance, there has been much confusion around the species delineation of *Claytonia* L. (Montiaceae) in Beringia since early exploration of the Arctic (Seemann 1857; Porsild 1974; O'Quinn and Hufford 2005; Miller and Chambers 2006; Elven et al. 2011). In an attempt to inform species level relationships, this project employed two approaches commonly taken when trying to distinguish species: a morphological approach and a genetic approach.

The morphological approach to species delineation is also referred to as the Linnaean or classical species concept (Burger 1975) and is the species concept of choice for many revisionary studies (Sokal 1973; Stuessy 2009). While there are multiple interpretations, all morphological species concepts require that species distinction be possible using phenotypic traits alone (Shull 1923; Sneath 1976; Stuessy 2009). So-called "alpha taxonomists" search for discrete lines to draw between groups of organisms while paying less attention to the biological processes that led to the formation of those groups (Cracraft 2000). This concept has been championed by giants in botany including Carl Linnaeus, and more recently Arthur Cronquist, who argued that species must be consistently distinct in such a way that they can be identified by ordinary means (Cronquist 1978). In *Flora of Alaska*, Eric Hultén relied almost entirely on morphological differences to distinguish species (Hultén 1968). While this approach is simple to follow in theory, it is also very subjective. The reliance on distinct and discrete differences between taxa means that species numbers may be highly exaggerated or significantly underestimated for groups that show significant morphological plasticity. Joseph Dalton Hooker, a contemporary of

Darwin, warned that the tendency of some to split taxa with every noticeable morphological difference would mean that every organism was considered a species (Stevens 1997).

Within Beringian members of *Claytonia*, significant morphological diversity is seen both between and within species. This study is a first attempt to quantify that morphological variation for *Claytonia* in Alaska using digital herbarium specimens and to identify discrete differences in morphology. My results successfully distinguished half of the taxa as distinct from others and provided discrete splitting criteria (Chapter 1). However, for more closely related taxa my morphological analysis failed to distinguish species. This may be in large part due to the measurements taken during my analysis and the fact measurements were aimed at trying to distinguish a wide variety of taxa, and not specifically geared towards deciphering relationships between closely related species. These measurements were aimed at capturing variation on a broader scale in hopes of deciphering major morphological differences within the genus rather than targeting morphological differences that would provide distinction between very closely related taxa. Additional data collection may provide more insight into recovering characters that might be able to delineate closely related species based on digital specimen images (Pereira et al. 2007; Sosa 2007; Rodrigues et al. 2013). However, if further morphological analysis is to be carried out, it will be important to sample species across the full geographic and morphological range (Young 1971; Stevens 1997). Steve Young, founder of the Center for Northern Studies and respected botanist of northern regions, cautioned against distinguishing a population as a new species if the trait being observed is continuous across its range (Young 1971). In his report on the flora of Noatak National Preserve, Young (1974) wrote about *Claytonia*, "*It is my belief that specimens from poorly known and relatively restricted geographical areas such as the Noatak study area are often 'overidentified' with regard to placement in infraspecific taxa. This is*

particularly true in the case of groups that have not been subject to detailed monographic treatment". While there has been a recent monographic treatment of *Claytonia* (Miller and Chambers 2006), many of the questionable taxa present in Alaska have not been addressed adequately and few specimens from Beringia were directly observed due to the remoteness of Alaska, and the inaccessibility of Beringia specimens from Russia. The Pan Arctic Flora (Elven et al. 2011) addresses some of the shortcomings of Miller and Chambers monograph (2006), including the fact that Russian distributions and treatments were not given much weight in the splitting of taxa. Thus the Miller and Chambers monograph (2006) often lumps many similar taxa into groups more frequently recognized by North American taxonomists.

Morphological analysis is just one approach to discerning species. This project also attempted to distinguish lower level taxa using a genetic approach. Within the last 50 years, genetic analysis has become the standard for determining relationships between taxa (Hillis 1987). The genetic species concept bases relationships between taxa entirely on the genotypic make-up of the organism (Stuessy 2009). Phylogenetic reconstruction to determine taxonomic relationships is now the modus operandi for systematists in most biological fields (Taberlet et al. 1991; Page and Charleston 1997; Soltis et al. 1997; Taberlet et al. 1998; Fedorov and Goropashnaya 2003; Shaw et al. 2005; Schuettpelz et al. 2006; Li et al. 2008; Chase and Reveal 2009). Now next generation sequencing makes it possible to compare entire genomes and base species relationships on whole-genome relatedness (Venter et al. 2004; Glenn 2011; Egan et al. 2012).

My study uses multiple quickly evolving markers of both the chloroplast and nuclear genome to elucidate phylogenetic relationships in Beringian *Claytonia*. Regardless of the ability of these markers to show lowest level resolution in other plant taxa (Baldwin 1992; Hamilton

1999; Li et al. 2008; Martirosyan et al. 2009), I was mostly unable to resolve species level relationships in this study. Adherence to a strict genetic species concept can result in organisms that are wildly variable morphologically being lumped into a single taxon if there is no genetic evidence for a split or, conversely, it can result in morphologically indistinguishable organisms being recognized as different species due to differences in genetic make up. Strict adherence to a genetic species concept in our study would mean the "lumping" of the most widespread and well-recognized species of *Claytonia* in Alaska including *C. arctica* Adams, *C. sarmentosa* C.A. Mey, and *C. scammaniana* Hultén. In contrast, previous studies using the circum-arctic *Saxifraga oppositifolia* shows significant divergence in *cp*DNA but very little morphological variation (Abbott and Comes 2004). Two subspecies of *Saxifraga oppositifolia* are now recognized based almost entirely on genetic variation.

There are many other species concepts that are regularly used to support relationships between taxa. These include a biological species concept that requires populations that are both genetically and geographically capable of interbreeding, an ecological species concept in which taxa are defined largely by their ecological niche, and an evolutionary species concept that goes beyond current genetic make-up and instead focuses on evolutionary lineages (Stuessy 2009). Scientists often debate species concepts as if they are the key to finding the answers when, in fact, real life can rarely be so cleanly and discretely categorized. In reality the species concept adhered to in a phylogenetic study or monograph is most often not even discussed (McDade 1995).

This project provided additional evidence for systematic relationships using two of the most frequently used species concepts. Both my morphological and molecular analyses provide support for currently recognized sectional divisions in *Claytonia* as well as additional insight into

the biogeographic history of this genus. However, my study did not produce clear, distinct relationships for all Beringian members of *Claytonia*. Perhaps different molecular approaches such as amplified fragment length polymorphisms (AFLPs), previously used for deciphering between closely related species in the Arctic (Schönswetter et al. 2007; Skrede et al. 2009; de Witte et al. 2012), or analysis of next generation sequencing could provide further resolution (Parks et al. 2009; Emerson et al. 2010; McCormack et al. 2013). Perhaps increasing the number of measurements and quantity of specimens used in morphological analysis would illuminate morphologically distinct taxa. My results demonstrate the recent divergence of Beringian members of *Claytonia* (inferred at 3.6 MYA) and suggest that species are still diversifying into individual niches reflecting low sequence divergence and incomplete lineage sorting. While species level resolution in this genus may require further study, our knowledge and understanding of relationships in this genus continue to improve and evolve as new evidence and analytical approaches become available.

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